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ANNUAL REPORT

OF

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NATIONAL INSTITUTE OF MENTAL HEALTH

Fiscal Year 1974

Volume II

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OF

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Fiscal Year 1974

Volume II

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MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research, and
Division of Biological and Biochemical Research

US NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1973 - June 30, 1974

VOLUME II

INDIVIDUAL PROJECT REPORTS

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ANNUAL REPORT
MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research, and
Division of Biological and Biochemical Research
NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1973 - June 30, 1974

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Serial No. M-OD-SW1
1. Office of the Director, DCBR
2. Section on Social Work
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Conflict Between the Parents of Schizophrenics

Principal Investigator: Carol F. Hoover, D.S.W. (Social Worker)

Cooperating Units: Section on Psychiatric Assessment.

Man Years:

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objective: To investigate levels of marital conflict, acknowledged anger and styles of coping with disagreement characteristically reported by the parents of schizophrenics (S), as compared to parents of maladjusted (M), and parents of community young people (C). Conflict over children, particularly index patient compared to his siblings and to the offspring of community families, is a particular focus. Marital dominance and perceived couple interaction are also studied.

Method Employed: A 70-item card sort, the Conflict in Marriage Scale (CIMS), is administered to each couple on two separate occasions a week apart. This procedure is scored to indicate conflict level and coping mechanisms. Marital disagreements which involve particular children are also reported.

Major Findings: A total of 154 individuals in the initial study were tested, either at the N.I.M.H. or their own homes. These included 30 couples from the community, located through city directories, 25 couples with maladjusted children, and 22 couples who had schizophrenic offspring hospitalized at the N.I.M.H. or elsewhere in the area. Each of these parent couples had two or more children between the ages of 13 and 27.

Data from the initial group of subjects has been reported in a doctoral dissertation, "Conflict Between the Parents of Schizophrenics," Catholic University, 1973. A shorter version of the material is being submitted for journal publication.

Same-sexed parental subjects could be divided into the indicated diagnostic groups (S,M,C) based upon responses to the CIMS items. Both M and S marriages reported more dissension than C partnerships. M children were embroiled in the most parental conflict. The schizophrenic patient was involved in significantly more conflict than his next-of-age sibling, but S parents reported less conflict involving their non-schizophrenic offspring than did C families. No across-the-board differences in marital dominance between the three groups were discovered, but responses to some items indicated that S wives felt themselves habitual losers, while S husbands did not experience themselves as victors in domestic combat.

A second group of eleven couples, parents of schizophrenics who met the same criteria, have now been tested, and this group is in process of analysis.

Serial No. M-AP(C)-14-10
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Evaluation of Family Dynamics with Conjoint Family Art Procedures

Previous Serial Number: Same

Principal Investigator: Hanna Yaxa Kwiatkowska

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0	
Professional:	0	(inactive)
Other:	0	

Project Description:

Objectives: The purpose of the project is (1) to evaluate families through a technique in which art media are used as a mode of communication and self-expression in order to compare (a) different families in which one or more of the offspring is afflicted with one of a variety of mental illnesses (schizophrenic, psychoneurotic, or delinquent) as well as normal families, and (b) different members within a family; (2) to evaluate through the same technique changes occurring in the course of family psychotherapy by repeating these procedures in periodic follow-ups during the treatment and after discharge.

Methods Employed: All members of a family are seen jointly in art evaluation sessions. The family is introduced to easy art media with no emphasis on artistic achievement. The structure of the session combines spontaneous self-expression with standardized procedures: (a) free picture, (b) family portraits, (c) abstract family portraits, (d) individual pictures started with the help of a scribble, and (e) picture done by the family jointly and started with the help of a scribble. These procedures are devised for the comparison of different families. The sessions are conducted by the art therapist with a cooperating investigator, a psychiatrist, or other staff member as participant observer. All the sessions are tape recorded, some of them video taped for a better study of family interaction. Abstracts of all sessions, describing the families' transactions, their comments, and discussions of their pictures, as well as special observations of the investigators, are dictated upon completion of the session; the art productions and tapes of the sessions are

studied for more accurate analysis. Each family is seen in a single evaluation session as close as possible after admission. Long-term patients and their families are seen in a second family art evaluation after a minimum of six months of conjoint family therapy or at termination of treatment.

In view of the principal investigator's not being officially connected with the project, work was concentrated on further analyses of the 1971-1972 findings on the investigated patient population. Observations on family dynamics with adolescents with behavioral problems, as seen in the Family Art Evaluation procedures, have been written up and will be included as a chapter of the book, "Family Art Therapy," commissioned by Charles C. Thomas, publishers.

Major Findings: The specific features observed in these families were as follows: A great deal of verbal and pictorial material was produced, but while the pictures were impressively revealing of the families' dynamics, the investigators were amazed at the difficulty these families had in recognizing the problems and interrelationships so vividly displayed. The quantity of material by itself overwhelmed the investigators, but even careful study of tapes rarely revealed a deeper engagement of the family.

In the joint endeavor (see Method (e) above), the fathers made ineffective efforts at leadership and were frequently sabotaged by the mothers. The joint production was usually of an inferior quality than the family members' individual ones.

The separation issue was repeatedly revealed in the pictures of the families of this same group of adolescents. These adolescents underlined in their family portraits their desire for complete separateness from the family unit, but upon closer investigation one discovers that there is an ambiguity in these representations: the adolescent pictures himself either outside of the carefully delimited world of the family, yet somehow still closely attached to it or in complete unreality such as hazy, drugged fantasy world or on a desert island.

On the other hand, the opposite tendency was noticed in the parents. The myth of happiness and unity of the family was predominant, with only hints at scapegoating the index patient. Their pictures underlined the unity of the family and encircled tightly the family world.

Significance to Mental Health Research: The development of this technique continues to give additional dynamic understanding of family relations to projects of the Adult Psychiatry Branch of NIMH. The hypotheses based on the empirical findings obtained through family art evaluations have been scientifically investigated by systematic computer analysis of the material and described as a separate project (M-AP(C)-14-12).

The researchers' and clinicians' interest in this technique continued to increase in the United States and abroad. As a result of the development of our work at NIMH, a graduate course in Family Art Techniques leading to a Master's Degree is offered by the Graduate School of Arts and Sciences at George

Washington University and at the Washington School of Psychiatry, and is taught by the principal investigator.

Seminars on family art techniques, in the United States and abroad, reprints of papers, and consultations continue to be requested.

Proposed Course of Project: The principal investigator continues work on a book dealing with the theory and practice of Family Art Therapy, mentioned earlier (under Methods Employed).

Honors and Awards: The principal investigator was awarded the Honorary Life Membership by the American Art Therapy Association for her pioneering work in the development of Family Art Techniques for clinical and research purposes.

Mrs. Kwiatkowska has presented, by invitation, numerous papers and conducted workshops on family art techniques. Among others: Graduate School of Social Work and Community Planning, University of Maryland, Baltimore; Graduate School of Social Work at The Catholic University, Washington, D. C.; Pratt Institute, Brooklyn, New York; Family Therapy Training Program, State of New York, Department of Mental Hygiene, Gowanda State Hospital; main speaker at the Annual Meeting of the Maryland Art Therapy Association, Baltimore, Md.

Serial No. M-AP(C)-14-11
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Family Art Therapy

Previous Serial Number: Same

Principal Investigator: Hanna Yaxa Kwiatkowska

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0	
Professional:	0	(inactive)
Other:	0	

Project Description:

Objectives: (a) To further investigate the pilot study where family art therapy is used as the sole therapy with families of adolescents from different diagnostic groups; (b) to use this approach with families of different socio-economic backgrounds.

Methods Employed: All members of a nuclear family are seen jointly in family art psychotherapy by the art therapist and the collaborating psychiatrist. Art media are offered to the family as a means of communication and self-expression; artistic or aesthetic considerations are not emphasized. In some of the sessions (at the beginning of treatment and repeated every six months), art evaluation procedures are used. These procedures combine free expression with standardized tasks and their purpose is to gauge the course of therapy periodically. Otherwise the family is largely left to structure the session; they are encouraged to draw or paint whatever comes to mind. All sessions are tape recorded; abstracts describing the family's interaction and the analysis of the pictorial and verbal material, as well as the therapists' subjective reactions, are dictated by both therapists. In the course of therapy, at least one session is video taped.

Major Findings: No family was investigated this year. The study based on a "blind" analysis by eminent psychiatrists and analysts of pictorial material obtained from long-term family art psychotherapy and chronologically arranged, was completed and presented last year, by invitation, at the American Psychiatric Association meetings under the title, "Blind Evaluation of Pictures in

Family Psychotherapy: Their Communicative Power," and demonstrated: (1) the blind judgments drawn from pictures only, without any verbal content were astonishingly accurate as to identity of index, diagnosis course, and outcome of therapy; and (2) the observations of the judges brought more clarity on the reasons for a successful achievement of the therapy and helped the therapists gain a more precise understanding of their experience with the family.

Proposed Course of Project: (1) To include the above study in a book dealing with the theory and practice of Family Art Therapy, commissioned by Charles C. Thomas, publishers, and (2) to complete for publication the monograph, "The Use of Drawing and Painting as a Primary Mode of Communication in the Family Therapy of Schizophrenia."

Honors and Awards: The Principal Investigator was awarded Honorary Life Membership of the American Art Therapy Association, for her pioneering work on the development of Family Art Techniques for clinical and research purposes.

Serial No. M-AP(C)-14-12
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Systematic Analysis of Family Art Evaluations

Previous Serial Number: Same

Principal Investigators: James K. Dent, Ph.D. and Hanna Yaxa Kwiatkowska

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.01
Professional:	0.01
Other:	0.00

Project Description:

Objectives: The overall objective is to explore systematically the materials available from the Family Art Evaluations which have been introduced by Ms. Hanna Kwiatkowska and conducted for several years. Such an analysis is aimed at improving diagnostic procedures and increasing our understanding of the social psychological dynamics of psychopathology. In presenting the specific objectives, the longer range of objects are listed first; the immediate objectives are listed last.

A. Family Dynamics and Psychopathology.

1. To differentiate, through the characteristics of their art productions, families in which the index is diagnosed as process schizophrenic, reactive schizophrenic, psychoneurotic, and so forth.

2. To distinguish role positions in the family (father, mother, sibling, etc.), as revealed by their pictures (such as family portraits).

B. Diagnostics (A Manual for the use of the Family Art Evaluations).

1. To specify the relation of the diagnosis of the index to the characteristics of his own art productions and those of his family.

2. To determine which characteristics of the art productions appear to represent transient symptoms and which represent more enduring personality characteristics.

C. Measurement Objectives.

1. To develop objective rating systems for pictures and family transactions in the Family Art Evaluation method.

2. To determine the interrater agreement and the internal consistency of the scales used.

3. To construct by combining scales, variables having stronger metric power.

4. To analyze the significance of variables like artistic talent and intelligence which may confound the relation between diagnostic variables and characteristics of the art productions. It is important to separate the evidence of psychopathology in a picture from the inference of intelligence or artistic talent.

Methods Employed: The pictures are blinded, randomized, and rated; one picture in four is check rated for studies of interrater agreement. A variety of statistical techniques are being used, depending upon specific objectives.

"Blind" ratings of pictures represent an independent measure of the mental status of an individual at a particular point in time. It is our plan to correlate these ratings with other data. Correlation with diagnosis and family characteristics such as socioeconomic status will be greatly facilitated because some of these data have already been compiled in connection with research done by other investigators in the last decade in this Branch.

Major Findings: Factor analysis across pictures reveals that there are some expected dimensions which are prominent and stable. For example, there is a dimension that might be labeled "bizarreness" which is always first or second in explaining variance regardless of how the factor analysis is conducted. However, there are many more independent dimensions than was anticipated; that is, even though interrater agreement is high, relations among dimensions tend to be low. The characteristics of the pictures are not easily summarized in a few dimensions.

Factor analysis across individuals indicate that there are a few dimensions which might be labeled "trait" dimensions. They extend across the various procedures, across two F.A.E.'s, and are correlated with intelligence and artistic talent. An example of such a dimension is the use of color. However, unexpectedly, "bizarreness" is not such a dimension; that is, an individual who produces a bizarre "first free" picture is not more likely to show "bizarreness" in his last picture.

There are a large number of dimensions which show significant differences between patients and siblings. These differences are almost always as one would predict; for example, patients' pictures are more likely to be "bizarre." Comparisons between offspring and their parents are difficult because the parents tend to show less artistic talent. Further analyses of talent-free dimensions are required.

Significance to Mental Health Research: Since the art production is an independent, enduring representation of the mental status of an individual (or family) at a moment in time and in a structured family setting, it is a valuable datum both for enhancing our diagnostic methods and for investigating family dynamics in relation to mental disorder.

The usefulness of "drawing tests" for individual diagnosis is widely recognized. Considerable research has been done on the Draw-A-Person test. More recently there have been efforts to use other types of art productions. Although the literature is full of ideas about how to diagnose mental illness from pictures, most of the research has been concerned with a much simpler and less useful task, viz.: To predict whether a person is mentally ill. Only a few systematic studies (e.g., those of Dr. Robert Kaye) have been concerned with trying to specify what aspects of a picture are predictive of what kinds of mental disorder, and there have been no statistical studies of family dynamics as seen through the art productions of the whole family.

Proposed Course of Project: Further analyses of diagnostic groups will be conducted.

Honors and Awards: None

Publications: None

Serial No. M-AP(C)-14-13
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Systematic Analysis of Brazilian Family Art Evaluations: A Replication.

Previous Serial Number: Same

Principal Investigators: Hanna Yaxa Kwiatkowska and James K. Dent, Ph.D.

Other Investigators: Dr. Carlos Paes de Barros, Dr. Aroldo Rodriques, Vera Pollo Flores, Vera Lemgruber Garcia, Junia Novaes.

Cooperating Units: Institute of Psychology, Catholic University, Rio de Janeiro, Brazil; Department of State Committee on International Exchange of Persons (Fulbright Commission).

Man Years:

Total:	0.01
Professional:	0.01
Other:	0.00

Project Description:

Objectives: The overall objective is to explore the differences and similarities appearing in the systematic analysis of pictorial material obtained from Brazilian families with the material obtained from American families here, in the identically structured situation of the "Family Art Evaluation." The specific objectives are:

1. To investigate and compare the characteristics of pictures drawn by Brazilian families where a family member has a specific psychiatric diagnosis, with the characteristics of pictures of diagnostically similar families in the United States.

2. To define if eventual differences observed through this analysis could be attributed to cultural variations.

3. To study the differences in roles of nuclear and extended family members in both countries, as seen in the different procedures of the Family Art Evaluations.

4. To investigate and compare the interrater agreement on the same items of the rating system in the United States and Brazil. This could also shed light on cultural differences.

Methods Employed: The manual used for the NIMH study was translated into Portuguese by the Staff of the Institute of Psychology at the Catholic University in Rio de Janeiro. It was edited by Hanna Yaxa Kwiatkowska; staff members were personally trained by her to conduct a study parallel to the NIMH study.

A psychologist, Ms. Vera Pollo Flores, with the consultantship of Ms. Sylvia Beatriz Machado Joffely, is in charge of the study and under the direction of Dr. Carlos Paes de Barros, Chairman of the Department of Psychology. Other staff members of the Institute were designated as coordinators and trainers of the raters. The ratings were done by students as part of their regular curriculum for credit. Dr. Aroldo Rodriques, Research Psychologist, is a consultant for the statistical aspect of the study. Another staff member of the Institute, a psychologist, Vera Lemgruber Garcia, personally visited us at NIMH in order to discuss the study.

The patients referred to the Clinic at the Institute of Psychology are mostly children or adolescents in their early teens. Jointly with their families they all have a Family Art Evaluation as part of their routine psychological work-up. A considerable amount of material has been accumulated since the evaluations were introduced there in 1966. In selecting sample families for the present study we were, nevertheless, limited by the age group; we do not want to include families where the index would be less than ten years old. Diagnostically the sample includes families of severely neurotic or behavior problem children, all outpatients at the Clinic. Records of all investigated Brazilian families were obtained and translated from Portuguese into English in order to obtain data (symptoms, onset, premorbid history) to be used to establish diagnoses of the Brazilian subjects.

Major Findings: Factor analyses reveal that there are some similarities with, and some differences from, the factor structure of pictures drawn by our families here at NIMH. The clarification of omissions and discrepancies in the Brazilian data was helped by the assistance of Ms. Junia Novaes, psychologist, guest worker at the Adult Psychiatry Branch, NIMH, who secured additional data while on a trip to Brazil in 1973. It was possible to fill in most of the gaps in communication which were necessary to finish statistical analyses. The first objective described above is complicated by the fact that most Brazilian indexes are outpatients, and few of the severe disorders in our NIMH patients are found among the Brazilian sample.

Significance to Mental Health Research: Family Art Evaluations provide a situation which offers a good deal of stability in the manner material is gathered for comparative studies. The basic material (pictures) is collected in an experimental setting structured as identically as possible with the NIMH setting. The methods used for the computer analysis of these data are also identical. The possible flaws in the translation and interpretation of the scoring manual, whether of linguistic or cultural nature, are being investigated in a separate study (M-AP(C)-14-14).

Proposed Course of Project: Final completion of analyses and publication of results. The Brazilian group plans to elaborate and refine the data in Brazil. The Principal Investigator, Ms. Hanna Yaxa Kwiatkowska, is being officially invited to Brazil to supervise further research.

Serial No. M-AP(C)-14-14
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Sources of Variance in the Crosscultural Application of an Objective System for Analyses of Pictures Drawn by Patients and Their Families

Previous Serial No: Same

Principal Investigators: James K. Dent, Ph.D., and Hanna Yaxa Kwiatkowska

Other Investigators: Dr. Carlos Paes de Barros, Dr. Aroldo Rodrigues, and Vera Pollo Flores

Cooperating Units: Institute of Psychology of the Catholic University of Rio de Janeiro, Brazil

Man Years:

Total:	0.01
Professional:	0.01
Other:	0.00

Project Description:

Objectives: A system has been developed for objectively classifying pictures drawn by patients and by members of their families in the Family Art Evaluations at NIMH (M-AP(C)-14-12). This rating system, which consists of about fifty dimensions, has been translated into Portuguese and is being used to describe pictures drawn by mental patients and their families in Brazil (M-AP(C)-14-13). In application, the two measurements may not be equivalent for several reasons:

(a) Various pictorial symbols may have different significance in culture A (American) from those in culture B (Brazilian).

(b) The English and Portuguese translations may not be equivalent.

(c) Research group A may have particular frames of reference not common to their own culture.

(d) Research group B may also have particular frames of references.

The objective of this project is to begin to sort out some of these various sources of error and variance.

Methods Employed: Sixty-eight pictures selected at random from about one thousand pictures (American families) have been or are being rated as follows:

Ratings 1 and 2:

Two members of research group A have independently rated each of the pictures using the English translation.

Rating 3:

These raters discussed their differences and prepared a check rating representing the best judgment of research group A for each picture.

Rating 4:

Using the English translation, the pictures have been rated by two American college student volunteers working alone (no contact with the research group), each doing about half of the pictures.

Rating 5:

Using the Portuguese translation, a bilingual American college student volunteer rated the pictures alone (no contact with the research group).

Ratings 6, 7, and 8:

These are like ratings 1, 2, and 3, but are being done by Brazilian members of research group B in Brazil, from slides of the same pictures rated at NIMH.

For the fifty dimensions in the rating system, comparison among these ratings should permit us to isolate translation difficulties and some of the group effects. Presumably differences not attributable to these two sources of variance represent cultural factors in interpretation or individual rate biases. (A study of individual rater biases is included in M-AP(C)-15-8, 1970-1971.) Because of the small number of pictures and of having only one bilingual rater, the results will have to be considered tentative. This project is essentially a pilot study for this type of investigation.

Major Findings: The Brazilians were able to rate only about half of the pictures sent them. Analysis of this half indicated clearly which dimensions have a constant meaning in both centers, which have different meanings but are reliable within centers (group frames of reference), and which are so poorly defined they have little meaning in either center. Ms. Junia Novaes, guest worker and graduate student from Brazil, has studied the various dimensions and has concluded that the differences between the Brazilian ratings and the NIMH ratings cannot be attributed to a lack of equivalence between the Portuguese and English versions of the rating systems.

Proposed Course of Project: Findings are being written up.

Significance to Mental Health Research: The precise description of mental disorders across cultures is hampered by a paucity of culture-free methods of assessment. Frequently the problem is dealt with by using the inferential judgments of particular clinicians who evaluate patients in two or more cultures. Agreement among them about particular patients is interpreted as constancy in measurement. This system - which is biased by language, dialect, and status problems - may not provide replicable descriptions; i.e., there are no assurances that another group of clinicians with differing backgrounds would provide the same description, or even a common one.

At the other extreme, one is impressed by the almost universal understanding and appreciation of works of art. The messages of enduring representations of art seem, on the one hand, to be an expression of the different cultures and, on the other hand, to convey emotions universally accessible. One is also impressed with the high quality of communication in pure science. Where ideas are precisely defined in observable terms crosscultural communication is facilitated. For these reasons objective descriptions of art productions may be an important way of achieving crosscultural communication in description of mental disorders. In addition to being tangible, the communication is not limited and/or biased by the language or dialect problem, and the professional has a direct access to the experience of the patient.

Honors and Awards: None

Publications: None

Serial No. M-AP(C)-14-15
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Intellectual Factors in Mental Disorders Among Adolescents

Previous Serial Number: Same

Principal Investigator: James K. Dent, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.01
Professional:	0.01
Other:	0.00

Project Description:

Part A:

Objective: To relate WAIS, IQ, and subtest patterns to diagnosis of mental disorders among adolescents.

Methods Employed: Data were obtained from the files of the Section on Clinical Psychology and are being analyzed by means of several multivariate techniques.

Major Findings: Preliminary findings indicate some consistencies with studies that are reported in the literature. Further analyses are needed to confirm these relationships.

Significance to Mental Health Research: Efforts to find WAIS patterns for various diagnostic groups have a long and somewhat discouraging history. It is generally believed that a major problem is the unreliability of diagnoses. The clinical and research records of the Adult Psychiatry Branch are a rich source of diagnostic material. For a number of years, in connection with this project and also M-AP(C)-14-12, the investigator extracted information to form relatively homogeneous symptom patterns. The result is a large number of groups with small numbers in each. However, this permits comparisons with other studies which have similarly defined narrow diagnostic groups. Preliminary findings are encouraging. Discovery of such patterns would (1) provide a relatively objective aid to diagnosis, and (2) shed light on intellectual factors associated with various disorders.

Proposed Course of Project: To continue plodding through a large number of previous studies that have been done in this area. It is expected that these findings will be written up during the next fiscal year.

Honors and Awards: None.

Publications: None.

Part B:

Objective: To relate WAIS, IQ, and subtest patterns to family role and mental disorders among adolescents.

Methods Employed: Same as Part A above.

Major Findings: The conceptualization of intellectual factors in mental disorders appears to shift radically when one passes from the individual to the family constellation. Although there is evidence of intellectual factors (positive intercorrelations) within families, still the important findings probably surround the constellations of factors rather than these homogeneities. All present analyses are quite preliminary.

Significance to Mental Health Research: There are probably very few research sites that have IQ measurements for whole families. Some work has been done on the IQ's of siblings of schizophrenics. But otherwise, practically nothing is known. In fact, a major problem has been to search out whether certain observed patterns for these families are also found among normals (e.g., the correlation between parents' IQ's).

Proposed Course of Project: Further searches of the literature are needed. It is doubtful that the investigator will have time to complete this work in the coming fiscal year.

Honors and Awards: None.

Publications: None.

Serial No. M-AP(C)-14-16
1. Adult Psychiatry Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Intellectual Factors in Unipolar and Bipolar Depressives

Previous Serial Number: Same

Principal Investigator: James K. Dent, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.01
Professional:	0.01
Other:	0.00

Project Description:

Objectives: To relate WAIS, IQ, and subtest patterns to diagnosis of unipolar and bipolar depressions. This project is similar to Part A of Individual Project Report M-AP(C)-14-15, except that these patients are adults. For these subjects, there is a large volume of other data that may be useful in understanding the patterns which are found. For example, performance on WAIS subtests is known to be related to certain measures of trait anxiety and of state anxiety in a complex interaction. For these patients, MMPI profiles are available; observational data are also available. The role of anxiety in these affective disorders is of considerable interest.

Methods Employed: Multivariate analyses of test data.

Major Findings: Substantial WAIS patterns are found for the two groups.

Significance to Mental Health Research: Efforts to find WAIS patterns for various diagnostic groups have a long and somewhat discouraging history. It is generally believed that a major problem is the unreliability of diagnoses. The research and clinical records of the Adult Psychiatry Branch are a rich source of diagnostic material. For a number of years, in connection with this project, and also M-AP(C)-14-12, the investigator extracted information to form relatively homogeneous symptom patterns. The result is a large number of groups with small numbers in each. However, this permits comparison with other studies which have similarly defined narrow diagnostic groups. Preliminary findings are encouraging. Differentiation of such patterns would (1) provide

a relatively objective aid to diagnosis, and (2) shed light on intellectual factors associated with various disorders.

Proposed Course of Project: To write up the findings, hopefully in the coming fiscal year.

Honors and Awards: None

Publications: None

Serial No. M-AP(C)-15-2
1. Adult Psychiatry Branch
2. Family Studies Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A Study of the Separation Process in Adolescents and Their Families

Previous Serial Number: Same

Principal Investigator: Helm Stierlin, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	.8
Others:	.8

Project Description:

Objectives:

The basic objectives of this study have remained the same as stated in the report of last year: to investigate, at the clinical and research levels, the individual and family factors which may further or jeopardize the separation process throughout adolescence. By tapping a large available reservoir of students with school problems, the study made use of an adolescent high risk population who, over the following five years, would or would not develop either in a psychotic, neurotic, or sociopathic direction. Understanding and possibly differentiating the separation course of these three patient groups was the main goal. This included a grasp of these patients' family and peer relations as they unfolded in time. The project built on many of the findings which Drs. Lyman C. Wynne and Margaret T. Singer and their associates reported during the last decade and a half. It addresses itself now to experimental factors which may allow certain high risk adolescents to steer clear of serious psychopathology while causing others, of seemingly equal or even lower risk, to succumb to such psychopathology.

Within the framework of this broad objective, the study tries, further, to throw light on several specific related issues. These are:

(a) Parental perceptions and expectations which specifically bear on their children's separation, e.g., their ability and willingness to seek partners outside the family or to take on responsible jobs. The investigator has become

increasingly impressed with the importance of such perceptions as factors which either inhibit or promote their children's moves toward autonomy.

(b) The individual and family dynamics of runaway adolescents. Twenty-six out of 43 index adolescents seen in this project had run away from home at least one full day and night, before they had turned 17 (some of them have run away frequently, getting as far away as Florida, California, and Canada). The project offered a unique chance to study in depth this group of runaways and thereby to better treat this large and important patient group.

(c) The often divergent developmental course of siblings who appear close in age and who at first sight appear exposed to similar family influences.

(d) The sequela of an early precocious and overstimulated development.

(e) Psychological and perceptual aspects of teenage drug abuse.

(f) The potential problems (particularly those relating to countertransference) and limits inherent in various treatment approaches - but mainly individual, couple, and family therapy - as used during a crucial juncture in an adolescent's life and separation process.

(g) The interlocking life crises of middle-aged parents and adolescent children.

(h) The role of the peer group in facilitating or hindering the adolescent's growth and separation.

(i) The similarities and differences between family myths and group fantasies.

(j) The roles of shame and guilt in adolescent separation.

(k) In continuing collaboration with Dr. Margaret T. Singer, Rorschach and TAT protocols of our families are being systematically studied. The main goal here is to transform the clinically derived concepts of transactional modes (i.e., the modes of binding, delegating, and expelling) into instruments for the evaluation and prediction of crucial adolescent separation dynamics which may also be applied transculturally.

Methods Employed:

Adolescents of age 15 and 16 who attend public schools in Montgomery County (Maryland) supplied the bulk of the patient material, in addition to those referred by private psychiatrists and various social and psychiatric agencies. The various referral sources sent adolescents who demonstrated school problems and whose families appeared willing to engage in family therapy. It was assumed that these students represented a high risk population both in the direction of schizophrenia and in the direction of sociopathic development. Mostly unbroken families were accepted. At the initial screening appointment, the family was seen as a group for 50 minutes and then the couple and designated patient were seen each for one-half hour. During the first two years of the

project, families were admitted only to outpatient therapy of at least three months duration. From the third year on, we began to accept inpatients also. During the fiscal years 1970 through 1972, we shifted exclusively to an inpatient program for the index adolescents which was gradually phased out during fiscal year 1973. Within the inpatient program, the patient was admitted to Unit 3-West where he received individual psychotherapy, usually three times a week. The family was seen in once- or twice-weekly family therapy sessions. Couple therapy once a week became mandatory. Individual psychotherapy for family members was offered when necessary and feasible. On the ward, the intensive therapy of the individual adolescent was supplemented by an intensive milieu program. No time limits were set to the individual or family treatment except for those dictated by the lengths of the therapists' stay at NIMH.

After the first screening interview the available historical, social, and dynamic data about the family were summarized and a fairly detailed prediction was made about the index patient. This prediction included ten major items such as employment in five years, psychiatric contacts and diagnoses, suicidal tendencies, delinquent tendencies, etc. The ensuing family and individual therapy was then used to obtain a more comprehensive picture of the family dynamics as they bore on the adolescent's separation problem and further development. The following areas were carefully observed and assessed:

(1) The prevailing parental perceptions and expectations, particularly insofar as these bear on the adolescent's separation.

(2) All data pertaining to runaway episodes of adolescents, e.g., overt or covert parental encouragement, the context of the running away episode(s), antecedents, peer contacts, etc.

(3) The communication patterns within the family. We tried to establish how much each family was bound to sabotage a pooling of resources.

(4) The nature of the family's alignments, their relative shifts and inconsistencies, their strengths, and their overt- or covertness.

(5) Family boundaries. We tried to assess how the family is integrated within the community and how much it might put obstacles in the way of the adolescent associating with peers, teachers, and other figures outside the family.

(6) Patterns of psychological exploitation: The various ways in which a child (more or less covertly) was recruited to serve his parents in ways which interfered with his own growth and separation.

(7) We tried to be alert to all data, implicit and explicit, which could throw light on the parents' roles in the adolescent's separation and, in particular, on their own separation problems when they were adolescents themselves.

(8) Through an actively and empathically conducted family therapy, we tried to get an indication of how much the family might be capable of changing its patterns and thereby facilitate the adolescent's separation. Also, by focusing

on the evolving transferences and countertransferences, we tried to get further clues about the parents' own separation problems.

On the basis of these assessments, which were supplemented by an individual interview with each family member, we made, at the end of the treatment period, second step predictions about the adolescent's prospective life style at the age of 21. These predictions covered the same items as those made immediately after the screening session, but they were now amplified. Also, we supplemented them by a narrative kind of prediction which dealt with the question of how much the index would become restricted or nonrestricted, active or more passive. In cases where there were two siblings closely related in age, the two of them were covered by predictions. It was expected that the latter confirmation, partial confirmation, or refutation of our predictions would deepen our insights and sharpen our hypotheses about the adolescent separation process. The comparison between the first step and second step predictions, as outlined above, appeared as a useful means of alerting us to possible sources of predictive error at this early stage of the research project.

While the principal clinical investigator tried to make five-year predictions based on the assessment of presumably crucial and lasting family features, as mentioned earlier, additionally obtained Rorschach tests, TAT, cognitive and perceptual style procedures of all family members, as well as the audio and video tapes, provided material for independent evaluations and predictions to be made either then or at later stages of the project. The availability of these test procedures made it further possible to utilize these families for other ongoing research projects of the Adult Psychiatry Branch, as described elsewhere.

Further, a standardized diagnostic inventory was made in accord with guidelines developed by Drs. John Strauss and Will Carpenter on all newly admitted index patients. This should provide us with a further means to check out (subjectively tinged) dynamic family data against other independently obtained and more standardized data.

Altogether 43 index patients and their families have been included in the program. An additional 19 families were seen in screening sessions only. These families, too, were made subject to predictions so that they could be followed up and could serve as untreated controls to the treated index patients and their families.

No new families were admitted during the last two years, and follow-up interviews were completed on 35 families. Eight families either refused to be interviewed or moved so far away as to make systematic follow-up impossible. Yet also on all these "refractory" families, we obtained at least some information. Most of the follow-up interviews were conducted in the families' homes. In these interviews, index patients, siblings close in age, and the two parents were seen for approximately 50 minutes each. The assessments directed to them covered the areas of earlier made predictions. Following these individual follow-up interviews, all family members were asked to listen to excerpts from audio or (when available) video tapes made at earlier conjoint family therapy

sessions and dating back up to five years. The family members were then encouraged to reflect together on how the situation conveyed by the tape excerpt did still exist and on how they all had changed or not changed. In the ensuing discussion there emerged often items and issues which had not been covered in the preceding individual follow-up interviews.

Major Findings:

In the systematic evaluation of the clinical material, described earlier, previous findings were consolidated and others emerged. The work of the last year yielded mainly these results:

(1) The conceptualizations on the separation process in adolescence, as outlined in last year's report, were extended, refined, and documented with more examples. Here the three transactional modes of binding, delegating, and expelling remained central, yet were reformulated so as to reflect vicissitudes of one basic delegating process which can derail in two major, but opposite ways - when the adolescent chafes under "missions impossible" that keep him bound up; or when he is sent out (i.e., pushed into premature autonomy), yet has no meaningful missions to fulfill. This new conceptualization was the theme of the Keynote Speech which the investigator gave at last year's World Psychiatric Association and The Australian and New Zealand College of Psychiatrists congress in Sydney, Australia.

(2) In line with this new conceptualization, differing dynamics and treatment implications with regard to schizophrenic, delinquency, and narcissistic developments were further spelled out and illuminated with examples. Here, the concept of "waywardness" proved particularly useful to distinguish expelled - i.e., neglected and rejected - delinquents from those who, through their delinquency, fulfilled vital missions for their parents.

(3) The findings on different types and family dynamics of runaways, as described in previous reports, were once more scrutinized with a view to establishing treatment principles. A paper which outlines these principles will be presented at a forthcoming joint session - in Detroit, Michigan - of the American Society for Adolescent Psychiatry and the American Psychiatric Association.

(4) The roles of shame and guilt in family relations were articulated within the above conceptual framework and documented with clinical material. Essentially, shame and guilt were here viewed as homeostatic forces that operate on various family levels and which can lock families into pathologic and almost unbreakable binds.

(5) The earlier described concepts on adolescents' separation dynamics were used to illuminate Adolf Hitler's family relations, his adolescent development, and his interactions with the German people. This involved the investigator in an extensive study of the, by now, enormous literature on Hitler. Several papers have been drafted that report here on the investigator's findings.

(6) There was, in cooperation with Dr. Margaret T. Singer, continuous work on devising and refining test instruments that would allow to make the above conceptualizations - and particularly the concept of transactional modes - more researchable, and hence would facilitate a new look at available data. Here the focus has been increasingly on TAT, rather than Rorschach, protocols as a potential means for a finer differentiation between the earlier described family scenarios and separation patterns.

Significance to Mental Health Research:

This study builds on findings about the structures, communication patterns, and boundaries of families with disturbed offspring which have been made in the Adult Psychiatry Branch during the past decade and a half. As time went by, it opened up ever new perspectives on the intergenerational separation process, in general, and the dynamics and treatment of schizophrenia and delinquency, in particular. Here the continuing focus on runaways appears commensurate with the increasing social importance of this patient group. A major effort is now made to translate major findings from this study into treatment principles and perspectives which may help not only mental health professionals, but also might directly assist the parents and adolescents concerned.

Proposed Course of Project:

While, as of this moment, the future of the Section is uncertain, it is hoped that a further year will be available for working on the rich materials this study has so far yielded.

Honors and Awards:

Appointed to the Advisory Editorial Board of Family Process.

First Prize winner of the 1973 Research Paper Award of the Washington Psychoanalytic Society, for the paper, "Psychoanalytic Approaches to Schizophrenia in the Light of a Family Model."

By invitation, member of panel, "Adolescence and the Disrupted Family," joint meeting of the American Psychiatric Association and the American Society for Adolescent Psychiatry, Honolulu, Hawaii, May 1973.

By invitation, held a seminar, "Schizophrenia and the Adolescent Separation Process," at the Child Psychiatric Clinic, University of Oslo, Norway, June 23, 1973.

By invitation, speaker and panelist at the International Congress of Psychotherapy, Oslo, Norway, June 25-30, 1973.

Invited lecturer, University of Heidelberg Psychosomatic Clinic, Heidelberg, Germany, July 6-7, 1973.

Invited lecturer, University Nervenklinik, Tubingen, Germany, July 1973.

By invitation, Keynote speaker and panelist at the World Psychiatric Association, in conjunction with The Australian & New Zealand College of Psychiatrists, Sydney, Australia, October 14-19, 1973. Investigator presented paper, "The Adolescent as Delegate of his Parents."

Invited speaker on nationwide television program, MONDAY CONFERENCE, the theme being "adolescence," Australian Broadcasting Commission, Sydney, Australia, Monday, October 22, 1973.

Invited lecturer, Australian Psychoanalytic Society, Sydney, Australia, October 23, 1973.

By invitation, seminar leader at The Family Therapy Institute, Department of Psychological Medicine, Monash University, Melbourne, Australia, October 25-30, 1973.

By invitation, Visiting Professor, lecturing and holding seminars, at Monash University, Melbourne, Australia, November 1-3, 1973.

By invitation, leader of symposium on adolescence at the Department of Child Psychiatry, Royal Children's Hospital, Melbourne, Australia, November 4-9, 1973.

Invited participant of the Advisory Editorial Board of Family Process at the Nathan W. Ackerman Memorial Conference, Cumana, Venezuela, February 6-13, 1974. Presented paper, "Shame and Guilt Binds in Families."

Invited speaker at the Washington Society for Adolescent Psychiatry, Washington, D. C., February 27, 1974. Presented paper, "Treatment Perspectives on Adolescent Runaways."

Publications:

Stierlin, H.: A family perspective on adolescent runaways. Arch. gen. Psychiat. 29: 56-62, 1973.

Stierlin, H.: Die Objektbeziehungen im Lebenslauf eines schizophrenen Vierlings. Psyche 27: 850-869, 1973.

Stierlin, H.: Group fantasies and family myths - Some theoretical and practical aspects. Family Process 12: 111-125, 1973.

Stierlin, H.: The adolescent as delegate of his parents. Aust. & New Zealand J. Psychiat. 7: 249-256, 1973.

Stierlin, H.: Psychoanalytische Ansatze zum Schizophrenieverstandnis im Lichte eines Familienmodells. Psyche 28: 116-134, 1974.

Serial No. M-AP(C)-15-6
1. Adult Psychiatry Branch
2. Family Studies Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Conceptualization of Lasting Dyadic Relationships

Previous Serial Number: Same

Principal Investigator: Helm Stierlin, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives:

This project seeks to contribute to the theoretical base for the comparative analysis of a wide variety of human relationships. It grew out of conceptualizations which the investigator developed before he joined the Adult Psychiatry Branch eight years ago and which were published in book form (Conflict and Reconciliation, New York: Doubleday-Anchor, 1969; Science House, 1969). The focus continues to be on the ongoing transaction between two persons who are considered as subjects - that is, as centers of an individual orientation toward the self, the world, and the other - and who are changed while the relationship unfolds. The phenomenology and dynamics of psychological exploitation appear here particularly significant. The implications and limits of a dyadic model for the better understanding of family and group relationships are being explored. Also, creativity as a response to a binding and conflictual interpersonal field and its relation to self-destruction remain a special focus of study.

Methods Employed:

Study, critical evaluation, and integration of a wide range of ideas and findings reported in the literature of human relationships. At the same time, the project draws on the clinical experiences and observations provided through the clinical service of the Adult Psychiatry Branch, and particularly through family therapies. Increasingly, the issues raised and described in Individual Project Report M-AP(C)-15-2 have influenced the here-described conceptual efforts.

Major Findings:

(1) The concept of psychological exploitation has been further developed and clarified and has been integrated with the concept of transactional modes. Specifically, it was related to how needs, power, and interpersonal justice evolve and are defined in human relationships.

(2) Various themes were pursued within the broad confines of this project. Among these were a study on how creative and self-destructive processes interweave, a comment on the psychogenic theory of history, a study on the psychoanalyst Paul Schilder, and several book reviews on diverse subjects.

Significance to Mental Health Research:

This conceptual work is relevant to the understanding of schizophrenia as a transactional thought disorder. Beyond that, it seeks to illuminate various mental disorders as being linked to special types of human relationships. The complexities of human relationships, it appears, have far outdistanced the conceptualizations so far at our disposal. This project tries to reduce the conceptual lag in this area. The concept of psychological exploitation seems relevant to many pressing social issues.

Proposed Course of Project:

The principal investigator is working on a book which pulls together the thoughts and themes developed under this project.

Honors and Awards:

Invited lecturer at the Symposium on Schizophrenia, "Schizophrenia and the Family: Research and Theory," sponsored by the Eastern Pennsylvania Psychiatric Institute, Philadelphia, Pennsylvania, April 12-13, 1973. Presented paper: "Bleuler's Concept of Schizophrenia in Modern Transactional Perspective."

Invited lecturer at the San Francisco Goethe Center, Branch of Goethe Institut (Munich, Germany), San Francisco, California, October 9, 1973. Presented paper: "Vicissitudes of Liberation and the Creative Process."

Invited panelist at the Center for Coördination of Anciety & Modern Studies Interdisciplinary Symposium, "Literature and Psychology," University of Michigan, Ann Arbor, Michigan, November 28-December 1, 1973.

Publications:

Stierlin, H.: Forward (Vorwort). In Mitscherlich, A. (ed.): Paul Schilder Entwurf zu einer Psychiatrie auf psychoanalytischer Grundlage. Frankfurt, Germany, Suhrkamp Verlag, 1973, pp. vii-xv.

Stierlin, H.: Interpersonal aspects of internalizations. Int. J. Psycho-Anal. 54: 203-213, 1973.

Stierlin, H.: Karl Jaspers' psychiatry in the light of his basic philosophic position. J. Hist. Behav. Sci. 10: 213-226, 1974.

Serial No. M-AP(C)-16-1
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: WHO International Pilot Study of Schizophrenia

Previous Serial Number: M-AP(C)-16-1, 16-3, 16-4, 16-7, 16-9, 16-13, 16-14

Principal Investigators: William T. Carpenter, Jr., M.D.
John S. Strauss, M.D., Co-principal Investigator,
University of Rochester School of Medicine and
Dentistry

Collaborating Investigator: John Bartko, Ph.D, and Alan B. Hawk, M.D.

Cooperating Units: Biometrics Branch, NIMH
University of Rochester Medical School
Prince Georges General Hospital
Spring Grove State Hospital
Morris Cafritz Memorial Hospital
World Health Organization and its Field Research
Centers in Colombia, Czechoslovakia, Denmark,
Great Britain, India, Nigeria, Taiwan, and Union of
Soviet Socialist Republics

Man Years:

Total:	2.6
Professional:	1.3
Other:	1.3

Project Description:

Objectives: (1) To develop standard interview and rating schedules that can be used cross-culturally for the evaluation of psychotic patients. (2) To evaluate a cohort of patients from the catchment area of Prince Georges County, Maryland with these instruments and to compare these findings with similar evaluations being carried out by investigators in eight other psychiatric centers in Colombia, Czechoslovakia, Denmark, Great Britain, India, Nigeria, Taiwan and the USSR. (3) To work toward a consensus with investigators from these other countries about criteria derived from the interview instruments, for classifying patients as schizophrenic. (4) To compare these WHO diagnostic evaluations with clinical evaluations and ratings of the same patients made with other methods such as the Lorr IMPS scale and projective tests. (5) To compare social and demographic data on patients evaluated by the standard instruments to understand more completely how these factors are related to schizophrenia as defined through the use of the standard diagnostic schedules. (6) To compare course of illness in patients from the collaborating centers by

conducting a follow-up study on patients seen in the original sample.

Methods Employed: Standardized forms have been developed for evaluating mental status, history, social function, and outcome. Initial evaluations were completed using these forms on 135 patients from each center. Data is being analyzed to compare the patients from the nine centers in terms of initial diagnosis, social function, and follow-up status. Alternative diagnostic techniques have been applied to evaluate their validity.

Analysis of variance techniques including the profile analysis of variance and stepwise discriminate function analysis have been used to identify discriminating features of schizophrenic diagnosis and the comparability of the schizophrenic diagnosis and subtype diagnoses across centers. Cluster analytic methods have been used in seeking new diagnostic groupings and an attempt to replicate subtype diagnoses commonly used.

Major Findings: Volume One of the IPSS report has been published. This report described methods and findings of the first phases of the IPSS. These phases include (1) the preparation, translation, pretesting, and revision of the data collection instruments; (2) the application of these instruments to 1,202 patients in nine different centers; (3) the evaluation of reliability of the interview schedules; and (4) diagnostic comparisons of the patients seen in the nine centers.

The preparation and translation of data collection schedules of this kind with collaboration of psychiatrists from nine centers is, in itself, a significant contribution to the methodology available to future cross-cultural studies. In evaluating the applicability and reliability of the schedules, two findings were especially significant. First, it was possible to use the same standardized mental status interview (in translation where necessary) in all of the collaborating centers and reliability of ratings within each of the centers was high. The reliability of the ratings across centers was at a lower but still acceptable level. Because history and social description data had to be collected in different ways in the different centers, and because these data depend so much for their psychiatric significance on the cultural milieu, it was more difficult to evaluate the comparability of these kinds of information from center to center. However, the schedules provided a useful means of collecting these data and represent a first step towards the development of comparable methodology in this area as well.

The comparison of similarities and differences in patient types seen in the different centers has been given considerable attention. The inherent complexity of such a comparison, even of patients within one center, is often underestimated, but was highlighted by the cross-cultural nature of this project. Three different methods for defining diagnostic types were used. The results of these three different methods were then compared. In the first diagnostic method, the interviewing psychiatrist assigned the patient to one of the commonly used clinical diagnostic categories. The categories defined by the International Classification of Diseases were those most frequently used; however, some centers tended to use special diagnostic categories. Analysis of symptom

profiles of the different diagnostic types showed broad similarities across centers for the general diagnostic categories such as schizophrenia, and manic-depressive psychosis. It indicated further that patients with some of the common defining characteristics of these diagnostic categories were seen in all centers. Comparison of symptom profiles to diagnostic labels applied by investigators from the centers also clarified many differences in the way in which psychiatrists from different centers used symptom information in reaching a diagnosis.

A second diagnostic method used to analyze the types of patients seen in this study was a computer program developed to simulate clinical diagnoses as they might be made by the psychiatric school led by Kurt Schneider. Using data from the mental status examination and psychiatric history, this computer method (Catego) was able to duplicate, to a large extent, the broad clinical diagnoses such as schizophrenia, and psychotic depression given to the patients by the investigators in the centers. This method represents considerable progress in developing a technique for applying diagnoses to patients which can be applied in different centers with complete reliability.

The third method used for assigning patients to diagnostic categories employed cluster techniques. With these mathematical techniques for defining groups of patients, it was possible to arrive at categorizations that were somewhat similar to both clinical and Catego diagnoses. The cluster techniques also provided a means for defining certain subgroups of patients that were seen in only one or two centers. The results of these three methods were compared and combined to describe basic similarities and differences among patients seen in the different centers.

Data from the IPSS is used in other projects previously described under M-AP(C)-16-3 and 4. Major findings have been (1) that Schneider's first-rank symptoms are discriminating of schizophrenia but fall short of the pathognomonicity claimed; (2) using Schneider's first-rank symptoms to increase the certainty of the schizophrenic diagnosis does not add predictability of poor outcome to the diagnosis of schizophrenia; (3) Langfeldt's diagnostic approach to schizophrenia has been found by a number of investigators to predict poor outcome. In this investigation, we are able to make a clean separation between discriminating symptoms and outcome and fail to find any evidence that Langfeldt's symptoms predict poor outcome; (4) the use of analysis of variance techniques has made it possible to determine the most discriminating symptoms of a schizophrenic diagnosis. A flexible diagnostic approach has been developed using the nine symptoms most discriminating for schizophrenia and three symptoms most discriminating of the nonschizophrenic psychiatric diagnoses. This system has the advantage of giving an estimate of the diagnostic error one could anticipate as well as being based on relatively few items of information that can be obtained with high reliability.

In addition to investigating signs and symptoms as diagnostic criteria and as predictors of outcome, we have used correlational, multi-regression, and discriminate function techniques to examine the relationship of prognostic variables with each other and their ability individually and collectively to predict outcome. The major finding is that they are relatively autonomous predictor outcome systems, so that past work performance predicts future work performance but is not a good predictor of overall outcome and past social adjustment predicts future social adjustment without being a good predictor of work function or general outcome. Past hospitalization predicts future hospitalization and can be generalized, to some extent, as a predictor of total outcome. Past hospitalization, past work performance, and past social adaptation have almost as much predictive power collectively as the entire 17-item prognostic scale.

The five-year follow-up on the IPSS patients is being conducted this year. At the time of this writing, we have outcome data on 60% of our original cohort. Preliminary data analysis has successfully replicated findings in the two-year follow-up demonstrating the poor predictive value of a schizophrenic diagnosis, whether made by DSMII criteria, Langfeldt's system, or Schneider's system. Paranoid-nonparanoid status does not predict outcome; neither does the degree of certainty of diagnosis based on our 12-point flexible diagnostic system. At the five-year point, the schizophrenic patients have a slightly worse outcome as a group than the nonschizophrenics. This is largely accounted for by the few patients with extremely poor outcome being placed in a schizophrenic group. An outcome scale has been further developed to include items on the patient's ability to care for himself and an estimate of "fullness of life." All items on the outcome scale have interrater reliability of .75 or better with the overall reliability being .97.

Significance to Mental Health Research and the Program of the Institute:
The difficulty in comparing psychiatric patients from different cultures has made it impossible to evaluate accurately the etiological importance of cultural factors in psychiatric disorder. The absence of standard methods for evaluation and diagnosis of psychiatric patients has also made it difficult to compare incidence and prevalence rated in different cultures and to compare usefulness of treatment methods employed in different centers. Until these comparisons can be performed, it is not possible to test many crucial hypotheses regarding the nature and etiology of psychiatric disorders. The difficulties in establishing standard instruments are considerable, not only in the writing and testing of such instruments, but in the extensive negotiations and communications necessary with the various investigators from different countries with diverse backgrounds and orientations. Nevertheless, in this study it has been possible to develop such instruments that can be applied in a useful way in a variety of cultural settings. These instruments were used with patients in nine different countries and proved to be applicable and useful for this purpose.

While methods of evaluating patient characteristics must be developed in order to perform cross-cultural studies, it is also essential to develop methods for categorizing patients once their characteristics have been elicited and rated

and to compare patient types among the different centers. Through applying these methods, it is possible to show in what sense patients seen in the different centers are similar and in what sense they are unique. The combinations of these methods can also be used to determine in what sense the statement, "schizophrenia is found in all of the nine centers," is true, and in what sense the statement omits recognition of real differences of "schizophrenic" patients from the different centers.

The two-year and five-year follow-up evaluations add a critical dimension to an assessment of schizophrenia. Finding a heterogeneous outcome cross-culturally of patients diagnosed schizophrenic and demonstrating the failure of accepted diagnostic systems to predict poor outcome provide a serious challenge to the postulate accepted in much of the world that a properly made schizophrenic diagnosis carries with it a poor outcome. Investigation of prognostic variables has challenged the confidence and the number of-criteria believed predictive of poor outcome and has suggested that relatively few items can be used for prognostication and each tends to predict future function in its own area rather than general function of the individual patient. Assessment of diagnostic criteria has challenged several systems but, most importantly, has provided an operationalized reliable system for identifying schizophrenic patients which is flexible enough to be adaptive to various settings and provides an estimate of the diagnostic error. Assessment of outcome is demonstrably complicated and the critique of this area has assisted in developing an outcome evaluation form which is easily applied and highly reliable.

Volume 2 of the IPSS Report has been drafted. It will be rewritten and go to press some time in 1974. Each of the nine Field Research Centers is presently completing collection of five-year follow-up data. Data analysis and writing of the joint report of five-year follow-up will commence during fiscal year 1975 and publication is anticipated late in 1975.

Data analysis currently under way in the Psychiatric Assessment Section is to evaluate subtypes of schizophrenia using profile analysis of variance and to determine if more effective subtyping can be accomplished through cluster analytic methods. Five-year follow-up data will be used in replication studies on several projects accomplished with two-year follow-up information.

With Dr. Carpenter's scheduled departure in July 1975, it is not anticipated that the Intramural Research Program will participate further in IPSS collaboration with the World Health Organization.

Publications:

World Health Organization (Eds.): International Pilot Study of Schizophrenia, Volume 1. World Health Organization Press, Geneva, Switzerland, 1973.

Strauss, J. S. and Carpenter, W. T.: The Evaluation of Outcome in Schizophrenia, to be published in Life History Research in Psychopathology, Vol. 3, M. Roff and D. Ricks (Eds.), University of Minnesota Press, Minneapolis, Minnesota.

Strauss, J. S. and Carpenter, W. T.: Characteristic Symptoms and Outcome in Schizophrenia, Arch. Gen. Psychiat. 30: 429-434, 1974.

Strauss, J. S. and Carpenter, W. T.: The Prediction of Outcome in Schizophrenia. II. Relationships between Predictor and Outcome Variables. A Report from the WHO International Pilot Study of Schizophrenia, to be published in Arch. Gen. Psychiat.

Carpenter, W. T., Strauss, J. S. and Muleh, S.: Are There Pathognomonic Symptoms in Schizophrenia?" An Empiric Investigation of Kurt Schneider's First Rank Symptoms, Arch. Gen. Psychiat. 28: 847-852, 1973.

Carpenter, W. T. and Strauss, J. S.: Cross-cultural Evaluation of Schneider's First Rank Symptoms of Schizophrenia: A Report from the International Pilot Study of Schizophrenia, to be published in Amer. J. Psychiat.

Carpenter, W. T., Strauss, J. S. and Bartko, J. J.: A Flexible System for the Identification of Schizophrenia: A Report from the International Pilot Study of Schizophrenia, Science 182: 1275-1278, 1973.

Carpenter, W. T., Strauss, J. S. and Bartko, J. J.: Use of Signs and Symptoms for the Identification of Schizophrenic Patients: A Report of the International Pilot Study of Schizophrenia, to be published in Schiz. Bull.

Bartko, J. J., Strauss, J. S. and Carpenter, W. T.: Similar Groups of Schizophrenia - Some Concepts and Findings, to be published in Schiz. Bull.

Strauss, J. S., Carpenter, W. T. and Bartko, J. J.: Towards an Understanding of the Symptom Picture Considered Characteristic of Schizophrenia: Its Description, Precursors and Outcome, to be published in Schiz. Bull.

Muleh, S. and Carpenter, W. T.: Book Review of Kurt Schneider's Clinical Psychopathology, (M. W. Hamilton, translator) (Grune and Stratton, 1959) in Compr. Psychiat. May-June, 1974.

Honors and Awards:

Dr. Alan B. Hawk presented a paper entitled, "Is Schizophrenic Outcome Dependent on Diagnostic Criteria?" at the Annual Meeting of the American Psychiatric Association, Detroit, Michigan, 1974.

Serial No. M-AP(C)-16-1

Dr. William Carpenter presented papers entitled "Are There Subtypes of Schizophrenia?" and "Borderline and Schizophrenic Patients: A Comparative Study" at the Annual Meeting of the American Psychiatric Association, Detroit, Michigan, 1974.

Dr. Carpenter made presentations on the diagnosis of schizophrenia, subtypes of schizophrenia, and outcome in schizophrenia at the following medical centers:

Dept. of Psychiatry, University of Rochester, Rochester, New York, 1973; Westchester Division of Cornell Medical Center, White Plains, New York, 1973; Annual Meeting of the American Psychiatric Assocation, Hawaii, 1973; Continuing Education Program, George Washington University School of Medicine, Washington, D. C., 1973; Dept. of Psychiatry, Washington Hospital Center, Washington, D. C., 1973; McLean Academic Conference, Belmont, Massachusetts, 1974; Division of Special Mental Health Research, NIMH, St. Elizabeths Hospital, Washington, D. C., 1974; Dept. of Psychiatry, Medical University of South Carolina; Dept. of Psychiatry, University of Chicago Medical School; Dept. of Psychiatry, Stanford University Medical Center, Palo Alto, California. Dr. Carpenter was the discussant on schizophrenia for Mental Health Matters, a NIMH radio program distributed nation-wide.

Serial No. M-AP(C)-16-5
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Serum Enzymes in Acute Psychotic States

Previous Serial Number: Same

Principal Investigators: William T. Carpenter, Jr., M.D.;
John S. Strauss, M.D.; Herbert Meltzer, M.D.

Cooperating Units: University of Rochester Medical School, Rochester, N. Y.
University of Chicago Medical School, Chicago, Illinois

Man Years:

Total:	0.2
Professional:	0.1
Others	0.1

Project Description:

Objectives: In an attempt to determine relationships between biochemical findings and psychotic conditions, Dr. Meltzer has demonstrated that creatine kinase and serum aldolase levels are often elevated in acute psychotic states. The objective of this project is to determine more precisely in which psychotic conditions these enzymes are elevated. By the use of standardized and diagnostic interviews to obtain information on a wide variety of symptoms and environmental conditions, it will be possible, using a sample of patients to define more clearly the correlations of individual symptoms with enzyme elevations. It will be possible in this way to evaluate how reliably these enzyme methods differentiate schizophrenic patients from other psychotic and borderline conditions.

Methods Employed: A series of patients is being interviewed using standardized mental status, history and social description interviews. Blood is drawn at the time of the mental state interview and analyzed employing methods currently in use by Dr. Meltzer for determining serum aldolase and creatine kinase.

Major Findings: An initial sample of patients has been tested with the techniques described and preliminary findings suggest that degree of "cognitive disorganization" as defined by a group of mental status items, correlates more closely than other parameters with elevated enzyme levels.

Significance to Mental Health Research and Program of the Institute: There is much interest in determining whether schizophrenia and other psychotic conditions can be related to particular biochemical and other physiological measures. Although there is some evidence for such relationships in regard to certain affective psychoses, evidence for similar relationships in schizophrenia is more controversial. Dr. Meltzer has been able to demonstrate relationships between creatine kinase and serum aldolase in psychotic conditions but it is not clear yet to what extent elevations in these enzymes are related to (1) the acuteness of the psychotic conditions and (2) particular clinical pictures. In the present study, it will be possible to help clarify some of these relationships as well as to evaluate by replication Dr. Meltzer's earlier work.

Proposed Course of the Project: A larger series of patients is being evaluated. On completion of the data collection, symptom patterns will be compared to the results of the enzyme determinations to see whether a meaningful relationship exists.

Serial No. M-AP(C)-16-6
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-HSMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychiatric History Study: The Nature and Reliability of the Data

Previous Serial Number: Same

Principal Investigators: William T. Carpenter, Jr., M.D. and John S. Strauss, M.D.

Other Investigators: Ruth E. Boesman, R.N.

Cooperating Units: John Bartko, Ph.D., Biometrics Branch, NIMH; Nursing Units 4E, 4W, 3E, 3W, NIH, Clinical Center; University of Rochester Medical School

Man Years:

Total:	:	0.1
Professional	:	0.07
Others	:	0.03

Project Description:

Objectives: (1) To identify different types of data ordinarily obtained in psychiatric history interviewing; (2) To study the reliability for these data as obtained from the patient compared to that obtained from his relative; (3) To study the effect of when in the course of hospitalization data is obtained on the nature of the data elicited; (4) To learn how psychiatric history is obtained in various psychiatric settings. These data will be used to examine the hypothesis that there is a greater variability in psychiatric history information than is usually expected and that the nature of the data obtained relates to the degree of variation.

Methods Employed: Standardized psychiatric history and social description interview schedules developed for the International Pilot Study of Schizophrenia were utilized in interviewing. The first 20 admissions to one of the psychiatric nursing units who also had relatives available as informants were interviewed. The patient and relative were interviewed within one week of admission and again six weeks later. The same interview schedules were used in each interview and the interviewing was rotated between the three investigators listed above in an effort to keep the interviewer as naive as possible to patient information other than that obtained during the interview.

A questionnaire was sent to a large number of facilities that admit psychiatric patients in the United States. This questionnaire requested information about who interviews whom and at what time to obtain psychiatric history information. Data has been collected from 20 patients and their relatives and is being analyzed to determine reliability. Narrative summaries of the interviews and certain key items are being examined by the investigators in order to determine comparability of the less structured aspect of psychiatric history evaluation.

Major Findings: Results show that data obtained by structured questions is most reliable. Several areas of data such as duration of illness, acuteness of onset and precipitating factors have low reliability. These findings challenge the validity of many concepts of psychiatric disorder that are based on results from unstructured collection of these data.

Significance to Mental Health Research and the Program of the Institute: The relationship of past experience to present behavior is fundamental to most psychological theories of behavior and mental function. There is a paucity of data testing the reliability and validity of background information obtained from psychiatric patients and their families. This study will attempt to identify the various means of collecting past history from psychiatric inpatients in various settings and to identify the pitfalls involved in interpreting this information.

Proposed Course of the Project: The findings are being prepared for publication.

Serial No. M-AP(C)-16-8
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychobiology of Cortisol Metabolism

Previous Serial Number: Same

Principle Investigator: William T. Carpenter, Jr., M.D.

Other Investigators: Edward B. Fink, M.D.

Cooperating Units: Section on Cognitive and Perceptual Studies, APB
Unit on Psychophysiology, LCP

Man Years:

Total:	.2
Professional:	.15
Other:	.05

Project Description

Objectives: To study the interaction between cortisol metabolism, behavior, and psychophysiologic variables in drug-free acute schizophrenic patients.

Methods Employed: Cortisol excretion will be estimated by determining urinary output of free cortisol and 17-hydroxycorticosteroids. Standardized clinical assessment techniques are used to gain detailed clinical information. Perception, cognition, and arousal are measured in collaboration with Drs. Zahn and Buchsbaum.

Significance to Mental Health Research and the Program of the Institute: Cortisol metabolism changes with mental state and behavioral changes. Furthermore, alterations in cortisol metabolism effect psychophysiologic responsivity. Thus, it is important to determine whether adrenal cortisol activity underlies or reflects behavioral disturbance, and which is the best predictor of psychophysiologic responsivity.

Proposed Course of Project: Studies of cortisol metabolism are being incorporated into the 4-East clinical unit's research program. In keeping with the major thrust of the work on 4-East, there will be careful attention to the clinical variables related to altered steroid metabolism and the possibility of using biological variables such as steroid metabolism as validating criteria

to test classification systems. The relationship between cortisol, psychopathology and psychophysiology is being investigated in collaboration with the Laboratory of Psychology.

Serial No. M-AP(C)-16-10
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Recovery Process and Research Data in Acute Psychosis

Previous Serial Number: Same

Principal Investigator: William T. Carpenter, Jr., M.D.

Other Investigators: Michael Sacks, M.D. and John S. Strauss, M.D.

Cooperating Units: Dr. Strauss--University of Rochester Medical School and
Dr. Sacks--Veterans Administration Hospital, New York City.

Man Years:

Total:	.2
Professional:	.18
Other:	.02

Project Description:

Objectives: (1) To define the difference phases of the psychotic process as the patient moves from severe decompensation toward recovery. (2) To describe the kinds of research investigations the patient is able to participate in as he passes through these phases, and the implications of this phasic process for interpreting results of research on psychosis. (3) To examine the relationship of the patients research participation to his therapy.

Methods Employed: Acutely psychotic patients were observed during the course of their illnesses as they participated or failed to participate in different research projects. These observations were supplemented by interviews with the patient, held prior to discharge, regarding his views on the research procedures.

Major Findings: Distinct phases of patient's illness have been identified. The three phases are: (1) The delusional phase during which patients typically were unaware of research expectations. Thought processes are fragmented and delusional. During this time the patient is unavailable for any kind of research or therapeutic activity that involves active cooperation with another person or cognitive engagement in a task. (2) Double-awareness phase during which the patient is still delusional, but has become aware of the ward research and therapy programs. A capacity for insight of his symptoms has begun and the patient begins to modify his behavior despite severe symptoms. Participation and cooperation in complex research tasks, although tedious, are now possible even though delusional interpretations of procedures may suggest a danger to the patient and, in fact, influence the way in which he takes the test.

(3) The recovery phase during which the patient is able to realistically perceive and evaluate the research and his participation in it comes with significant clinical improvement. When the patient fails to cooperate in research procedures, he has clear reasons for refusing -- which reflect his attitude toward the unit, his therapy and his doctor. Themes about the research which were present in delusional form during earlier phases may continue, but are now expressed more rationally in a therapeutic relationship. Research that can be carried out is restricted by the phase through which the patient is passing. In the more disturbed phase, no information is obtainable about either response or other measures requiring active collaboration of the patient; as the patient improves more aspects of his function can be evaluated.

Significance to Mental Health Research and the Program of the Institute:
The major focus of experimental research on psychosis in the past has often been on chronic patients. Only recently has greater attention been directed toward the acute patient. This study attempts to define phases of the acute process and to demonstrate their interrelationship to the research findings, the conclusions that can be drawn from them, and the patient's therapy. Identifying the psychological set during collection of data provides a framework for interpreting results which enriches the disease model or simple behavioral rating approach. Awareness of the patient's research experience is vital to the therapist since patients will often utilize their research participation to express thoughts and feelings that otherwise might not be available.

Publications:

Sacks, M. H., Carpenter, W. T. and Strauss, J. S.: Recovery from Delusions: Three Phases Documented by Patient's Interpretation of Research Procedures. Arch. Gen. Psychiat. 30: 117-120, 1974.

Serial No. M-AP(C)-16-11
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Investigation of the Schizophrenic Process Through Art Productions of Acutely Psychotic Patients

Previous Serial Number: Same

Principal Investigators: Harriet Wadeson and William T. Carpenter, Jr., M.D.

Cooperating Unit: 4-East Nursing Unit, Clinical Center, NIMH

Person Years:

Total:	1.0
Professional:	.9
Other:	.1

Project Description

Objectives: (1) To obtain art productions from acutely psychotic patients at intervals during the course of their hospitalization and follow-up. (2) To utilize the art productions in conjunction with other clinical assessments for the purpose of diagnosis and treatment planning. (3) To aid in the treatment of those patients for whom art expression is particularly beneficial. (4) To compare and study the art productions and the patients' associations to them in order to gain a greater understanding of the schizophrenic process by (a) investigating longitudinal changes by comparing pictures made during acute psychosis, recovery, and follow-up; (b) studying in detail delusions, hallucinations, and other manifestations of schizophrenic thinking as revealed in the art productions; (c) comparing art productions of different patients with one another in order to delineate subgroups of schizophrenic ideations; (d) contrasting the art productions of schizophrenic patients with those of manic and depressed patients.

Methods Employed: All patients on a research ward for the study of acute schizophrenia are evaluated in individual therapy sessions shortly after admission, just prior to discharge, and at follow-up. Some patients have additional sessions during the course of hospitalization if there is marked change in the patient's condition. Patients are provided with simple media and encouraged to express themselves freely and to free associate to their pictures. At each session, they are requested to make the following pictures: (1) whatever they want to draw; (2) a self-portrait; (3) a picture of the illness; (4) pictures of any hallucinations they have had; (5) pictures of any delusions they have had. If there are other pictures that patients particularly want to produce, either during the session or at any other time, they are

encouraged to do so and to discuss them with the investigator. To date 57 patients have been studied. Each session is recorded on audiotape and a summary is written by the investigator. All pictures are photographed and catalogued for study purposes.

Specific Studies: (1) Hypotheses have been developed and are in the process of being tested by means of determinations from blind raters relating differences in expressiveness in art to differences in recovery style from acute schizophrenic episodes. (2) Twenty-three variables have been teased out from over 700 pictures collected to date and are being studied in relation to clinical state, subgroupings of acute schizophrenics, course of illness and recovery, premorbid state, and outcome predictions. In addition, changes in style, content, self-image, directions of change and a consistency-variety continuum are being investigated in relation to course of illness and recovery.

(3) A further area of interest is the comparison with standardized verbal interviews of art expression as an investigative tool.

(4) Another study compares art productions of acute schizophrenics with those of psychotically depressed and manic-depressed patients, from whom the investigator has collected an abundance of material from past projects. Samples from patient groups have been matched for age and picture assignment and will be tested for differences and similarities in the pictures in expression of depressive feelings, confusion, and excitement. Other variables may be investigated as well. Particular attention will be paid to the often puzzling group of schizo-affectives from both populations.

(5) In the beginning stages is a project to study the subjective experience of psychosis as expressed in pictures with particular focus on its pleasurable and unpleasurable properties. Related to this is perception of cause of illness as believed to be external or internal to the self and as the result of physical or psychological influences. The data for these determinations is in the picture of illness made by each patient at admission, discharge, and follow-up.

(6) Data is being collected to determine phenothiazine effects by comparing pictures made by the same patients receiving medication and off medication within a limited time span. At present, data is being collected,, but the sample is still too small to begin to test hypotheses.

Major Findings: We have developed the first systematic collection of psychotic patients' graphic depictions of their hallucinations and delusions. A representative sample has been exhibited at the American Psychiatric Association Annual Meeting, the 7th International Congress of Psychopathology of Expression, the Annual Meeting of the American Art Therapy Association, and the U.S. Public Health Service Professionals Association Annual Meeting. At the request of the Japanese Journal of Art Therapy, a paper has been prepared on hallucinations and delusions which notes the

following findings: (1) incidence of occurrence of hallucinations and/or delusions in over 95% of our patients; (2) the fleeting nature of hallucinations compared with the more enduring experience of delusions; (3) prevalence of religious themes and transformations of real objects; (4) differences in hallucinatory and delusional experiences along variables of paranoia, grandiosity, organization of ideas, and conviction of the realness of the experience.

Significance to Mental Health Research and the Program of the Institute: There is little data of a comparative nature on the art productions of any particular population. Most of the writing in this area is directed toward therapeutic benefits rather than diagnosis and evaluation. Where such reports exist, they are primarily speculative and do not report characteristics of a designated population that has been studied systematically. The purpose of this project is to help counter this lack. In a broader sense, although the outward manifestations of acute schizophrenia are readily studiable (symptoms, progress, etc.), the inner experience of the affected individual is much less accessible. Hopefully, this investigation will provide data in this area. Finally, in conjunction with other parameters of assessment, it is hoped that the data obtained from the art evaluations will add to the clarity of differential diagnostic dimensions in acute schizophrenia as well as differences and similarities across diagnostic groups.

Proposed Course of the Project: It is anticipated that within the coming year all the studies outlined above will be completed. For only one (comparisons on and off phenothiazine) will it be necessary to collect additional data. Most are in the data analysis stage. In addition, work with the 23 variables will require the development of several formulations.

Since the nature of the data lends itself to systematic investigation not available in other art therapy settings, observations postulated elsewhere regarding changes in schizophrenic art expression over course of ego disintegration and reintegration will be tested using the data collected.

Publications

Wadeson, Harriet: Art Therapy Techniques Used in Conjoint Marital Therapy, Amer. J. Art Therapy, April 1973.

Wadeson, Harriet: Separateness. Art Psychotherapy 1: Fall 1973.

Wadeson, Harriet and Carpenter, William T., Jr.: Hallucinations and Delusions, exhibit and booklet, American Psychiatric Association Annual Meeting, Honolulu, 1973; 7th International Congress of the Psychopathology of Expression, Boston, 1973; American Art Therapy Association Annual Meeting, Columbus, Ohio, 1973; U. S. Public Health Service Professional Association Annual Meeting, Washington, D. C., 1974.

Contributions to Sarason, Irwin G.: Personality, an Objective Approach. John Wiley & Sons, New York, 1972.

Presentations:

"My Approaches to Art Therapy," Univ. of Md. School of Social Work, May 1974.
Presentation to Md. Assoc. of Art Therapy, April 1974.
"Research in Art Therapy" - panel discussion, Annual Meeting of the American Art Therapy Assoc., 1973.
"Couple Art Therapy" - demonstration, Annual Meeting of the American Art Therapy Assoc., 1973.
"Intrapsychic Effects of Amphetamine in Hyperkinesis as Revealed through Art Productions" - paper, 7th International Congress of the Psychopathology of Expression, Boston, Oct. 1973.
"Couples Who Draw Together" - Human Behavior 3(2), Feb. 1974.
"Mental Health Matters" - National radio program - March 1974.
Colloqium on Space, Movement and Expression, Goddard College, Washington, D. C., March 1974.
"Introduction to Art Therapy" Workshop, Community Services, Montgomery College, Takoma Park, Maryland, April 1974.
"Advanced Art Therapy" Workshop, Community Services, Montgomery College, Takoma Park, Maryland, May 1974.

Serial No. M-AP(C)-16-12
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Evaluation of Urinary Bufotenin and Dimethoxytryptamine (DMT) and Serum Methyltransferase in Acute Schizophrenia

Previous Serial Number: Same

Principal Investigator: William T. Carpenter, Jr., M.D.
Associate Investigators: Harold E. Himwich, M.D.
N. Narasimhachari, Ph.D.
Edward Fink, M.D.

Cooperating Units: Galesburg State Research Hospital

Man Years:
Total: .2
Professional: .1
Other: .1

Project Description:

Objectives: (1) In previous investigations, Dr. Himwich has consistently found hallucinogenic substances in the urine of schizophrenics, while absent in normal controls. More recently, his laboratory has found a probable transmethylating enzyme in the serum of schizophrenics, while absent in normals. The objective in this investigation is to test the hypothesis that schizophrenics abnormally transmethylate hallucinogenic compounds.

Methods Employed: Drug-free acute schizophrenic patients subjected to intensive clinical evaluations provide urine and blood samples for biochemical determinations. Nonschizophrenic patients and normal volunteers provide control urine and blood specimens. The schizophrenic patients are studied during sick, recovered and follow-up periods. Biological specimens are shipped frozen to Galesburg, Illinois for assay.

Psychiatric Assessment interview schedules are used in the evaluation of patients hospitalized for study at the Galesburg State Research Hospital. These chronic schizophrenic patients are taken off drugs for periods ranging from 6 to 12 weeks with the biologic investigations being carried out during this extensive drug holiday.

Major Findings: Hallucinogenic substances, bufotenin and dimethoxytryptamine, are found in the urine of acute and chronic schizophrenic

patients. A substance in serum which is probably a transmethylating enzyme is found in the serum of acute and chronic schizophrenic patients. However, we have now found urinary bufotenin and DMT as frequently in normal controls as in our schizophrenic subjects. These findings are contrary to those hypothesized and significantly weaken this aspect of the transmethylation hypothesis in schizophrenia. A transmethylating enzyme has been found in serum of nonschizophrenics by three other groups and this finding is replicated in our normal volunteers.

Significance to Mental Health Research and the Program of the Institute: These findings demonstrate the necessity of a rigorous clinical methodology in psychobiological research in schizophrenia. The refutation of previous work requires a reconsideration of hypotheses that have received considerable attention in recent years.

Proposed Course of Project: Data analysis is still in progress but data collection and the pursuit of the implication of these findings has been discontinued.

Honors and Awards:

Dr. Carpenter made presentations on psychobiologic investigations of acute schizophrenic patients to the Dept. of Psychiatry, University of Pittsburgh Medical School; Laboratory of Psychology, Intramural Research Program, NIMH; and Human Development Seminar at the University of Maryland.

Publications:

Narasimbachari, N.; Baumann, P.; Pak, H. S.; Carpenter, W. T., et al.: Gas Chromatographic-Mass Spectrometric Identification of Urinary Bufotenin and Dimethyltryptamine in Drug-Free Chronic Schizophrenic Patients, to be published in Biological Psychiatry.

Serial No. M-AP(C)-16-15
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Group Processes on Clinical Research Units

Previous Serial Number: M-AP(C)16-2

Principal Investigator: William T. Carpenter, Jr., M.D.

Associate Investigators: Michael Sacks, M.D. and Edward Fink, M.D.

Cooperating Unit: Dept. of Psychiatry, VA Hospital, New York City

Man Years:

Total:	.2
Professional:	.18
Other:	.02

Project Description:

Objectives: To identify and conceptualize group processes on a clinical research unit and examine their relationship to therapeutic and research tasks.

Methods Employed: Study group techniques have been used to make observations on ward functioning vis-a-vis therapeutic and research work tasks.

Major Findings: (1) The concepts of emergency and crisis have been defined and applied to a psychiatric unit. The crisis is said to exist when the unit is dominated by a covert work task. Evidence for crisis may be subtle but include the breakdown of ordinary work structures. For example, meetings fail to begin and end on time, absenteeism increases, cohesiveness between staff is lost, roll function becomes defuse, personnel are stereotyped, and therapeutic patterns, such as the giving of medication, are altered. Emergency is defined as a gross disruption of the unit requiring immediate management. Aspects of crisis recognition and emergency prevention are discussed in the publication listed below. (2) Conceptualized from a group-process orientation, five prominent, anti-therapeutic trends have been identified. They are (a) the absence of a therapeutic standard; (b) assignment of irresponsibility; (c) establishment of an anti-therapy leader; (d) the absence of therapeutic leadership, and (e) maintaining a pathogenic environment (the community double bind). (3) Emphasis on defintion of a work group task and the organization structure for pursuing it are relevant to successful collection of psychobiological data in acute schizophrenic patients. In addition to individual behavior based on

intrapsychic conflict, we found that group expectations are perhaps the most important determinant of a patient's behavior vis-a-vis research expectations. Increasing attention to group phenomena as related to the research endeavors was accompanied by increasing success in patient participation in a variety of psychobiological investigations. Individual patient differences do not account for the increased success. (4) Informed consent is a difficult concept applied in any medical setting and special complications in doing research with schizophrenic subjects have been noted. An approach to informed consent which has some success in engaging the patients and advocating on their own behalf is described in the publication listed below.

Significance to Mental Health Research and the Program of the Institute: The observations and concepts related to these projects have general applicability across psychiatric units and probably on most medical units. Enhancing the recognition of the crisis is fundamental to the prevention of an emergency. Awareness of important anti-therapeutic tendencies on psychiatric units provides an opportunity to enhance the consistency with which a unit can accomplish its therapeutic work. A new approach to informed consent provides and opportunity for fuller participation by the patient in understanding the risk and benefits and his roll in investigative and/or therapeutic studies. Increased sensitivity to the relationship between organization and work task provides an opportunity for enhancing psychobiological investigations of acutely psychotic patients.

Honors and Awards:

Dr. Sacks presented a paper on the anti-therapeutic community at the Eighth Joint Meeting of the Professional Association of the U.S. Public Health Service in Phoenix, Arizona, 1973 and a paper entitled, "Crisis and Emergency on the Psychiatric Ward," at the 4th Multistate Interhospital Conference of the Eastern Psychiatric Research Association in New York City, 1973.

Dr. Carpenter made presentations on the research milieu and treatment of schizophrenics to the Dept. of Psychiatry at Yale University, New Haven, Connecticut and the Dept. of Psychiatry, Medical University of South Carolina.

Publications:

Sacks, M. H., Carpenter, W. T., Jr. and Scott, W. H.: Crisis and Emergency on the Psychiatric Ward, Comprehensive Psychiatry 15: 79-85, 1974.

Sacks, M. H. and Carpenter, W. T.: Anti-therapeutic Forces on Psychiatric Units, to be published in Hospital and Community Psychiatry.

Carpenter, W. T., Jr.: A New Setting for Informed Consent, The Lancet 1: 500-501, 1974.

Serial No. M-AP(C)-16-16
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: CSF amine metabolites in acutely schizophrenic patients

Previous Serial No.: None

Principle Investigator: Edward B. Fink, M.D.

Other Investigators: William T. Carpenter, Jr., M.D.
Robert M. Post, M.D.
Frederick K. Goodwin, M.D.

Cooperating Unit: Section on Depression, APB
Section on Psychiatry, LCS

Person Years:

Total:	.25
Professional:	.15
Other:	.10

Project Description

Objectives: (1) To utilize the probenecid technique to study dopamine, serotonin, and norepinephrine metabolism in a drug-free, acutely psychotic patient population; (2) To restudy the same patients when they are recovered; (3) To investigate the relationship of the measures of amine turnover to standardized ratings of signs, symptoms, premorbid factors and outcome.

Methods Employed: Consecutively admitted schizophenic patients are studied during acute psychosis after a minimum of 2 weeks off medications. A baseline LP is performed at 9:00 a.m. after 9 hours bedrest, followed by probenecid administration (100 mg./kg.) over 18 hours, after which a second LP is performed at 3:00 p.m. During a drug-free, recovery period about 4 months after admission, the baseline and post-probenecid LP's are repeated in identical fashion. An 8 cc CSF specimen is assayed by photofluorometry for serotonin and dopamine metabolites, 5HIAA and HVA, and by gas chromotography for the norepinephrine metabolite 3-methoxy-4-hydroxyphenyl glycol (MHPG). Data is systematically collected regarding daily clinical symptomatology, premorbid psychiatric, social, and work history, family history of mental illness, and outcome.

Results from 17 patients reveal that mean values for baseline and probenecid-induced accumulation of 5HIAA (28.4 $\pm$ 2.6 and 145.1 $\pm$ 14.3, and

HVA (21.2 ±2.3 and 248.6 ± 28.9) are not significantly different from comparably studied normal controls (baseline only) and affective disorder patients. Mean baseline MHPG (17.7 ± 2.3) is not significantly different from the normal and manic groups, but the previously reported depressed group had lower MHPG.

Correlation coefficients revealed no significant relationship demonstrated between levels of 5HIAA, HVA, or MHPG and the presence or severity of symptoms or premorbid and family history items.

To date four patients have been studied during both admission and recovery test periods. Paired t-test revealed significantly lower probenecid-induced HVA accumulation ($p < .05$) and significantly lower baseline MHPG ($p < .025$) during recovery phase testing.

Significance to Mental Health Research and the Program of the Institute: Among the several important biological hypotheses which attempt to account for schizophrenic symptoms are those postulating altered CNS amine metabolism. Amphetamine psychosis and phenothiazine receptor blockade implicate dopamine, the 6-hydroxydopamine self-stimulation model emphasizes norepinephrine, and the effects of LSD, PCPA, and DMT implicate serotonin. Despite the serious shortcomings of each of these as a model for schizophrenia, they deserve clinical investigation.

The above data for baseline and probenecid-induced determinations in drug-free, acute schizophrenics suggests that dopamine, serotonin, and norepinephrine metabolism is not significantly different from healthy or affective illness subjects. Preliminary evidence suggests that changes in amine metabolism do take place after recovery from the acute episode. It is possible that the reductions in HVA and MHPG after improvement indicate that, for a given patient, the levels observed during acute schizophrenia are elevated and reflect altered CNS catecholamine neurotransmission. Whether such an abnormality is of etiological significance, or an epiphenomena of an acutely disorganized psychotic state, or whether an underlying amine deficiency predisposes one to psychosis, should be further clarified by the continuing investigation of our patient population.

Proposed Course of the Project: Following the above protocol, data continues to be collected from patients on 4-East and 3-West during both admission and discharge test periods. It is expected that the increasingg number of patients in the study will help to clarify the significance of the present findings which suggest that within-patient changes in amine metabolism are dependent on clinical state.

Honors and Awards:

Dr. Fink presented a paper entitled, "CSF Amine Metabolites in Acute Schizophrenia," at the New Research Reports, American Psychiatric Association Annual Meeting, Detroit, 1974.

Serial No. M-AP(C)-16-16

Dr. Carpenter made presentations on psychobiologic investigations of acute schizophrenic patients to the Dept. of Psychiatry, University of Pittsburg Medical School; Laboratory of Psychology, Intramural Research Program, NIMH; and Human Development Seminar at the University of Maryland.

Serial No. M-AP(C)-16-17
1. Adult Psychiatry Branch
2. Psychiatric Assessment Section
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Integration and Sealing-over: Clinically Distinct Recovery Styles from Schizophrenia

Previous Serial Number: None

Principal Investigators: Thomas H. McGlashan, M.D.
Steven T. Levy, M.D.
William T. Carpenter, Jr., M.D.

Man Years:

Total:	.4
Professional:	.38
Other	.02

Project Description:

Objectives: To develop and define the concepts of integration and sealing-over as distinct recovery styles from acute psychotic episodes through clinical observations of the recovery process and, using these definitions, to classify patients into integration and sealing-over groups for comparison using multiple clinical and biological indices.

Methods Employed: The data arises from our therapeutic and research interactions with acutely schizophrenic patients from the 4-East Clinical Research Program. Judgments dichotomizing patients into integration or sealing-over subgroups were made by the patient's psychiatrist and two research psychiatrists using interviews and medical records. At one-year follow-up interviews patients discussed their psychotic experience and were again rated. Ten matched patients were selected for each group (integration and sealing-over). Using independently collected behavioral and biological data, similarities and differences between the groups were determined using analysis of variance and discriminate function techniques.

Major Findings: Patients can be reliably assigned to each group. Operational definitions of integration and sealing-over have been formulated. Integrators are more curious about their symptoms, regard them as part of their life's pattern, and gain information about them, resulting in a more flexible and variable attitude towards illness than patients who sealed-over. The latter had fixed views of their illness (generally negative), did not understand their psychotic symptoms, nor did they regard their psychotic experience

as a personal expression. Empiric analysis discriminated the two groups on seven variables which suggest that patients who integrate may have better perceptual-cognitive control and may be less overwhelmed by aberrant internal and external stimuli. These results support the validity of integration and sealing-over as defined and help to refine our understanding of the schizophrenic patient's response to his own psychosis.

Significance to Mental Health Research and the Program of the Institute: Little research attention has been directed to a patient's recovery style. Integration and sealing-over are important concepts in this context and can be applied to treatment philosophies. For example, the psychiatrist who views psychotic symptoms as the manifestation of organic disease best treated by pharmacotherapy may facilitate sealing-over but impede the integrative process. Likewise, a psychiatrist invested in the psychotherapy of schizophrenia may impede sealing-over in patients so predisposed. Defining and operationalizing these concepts may facilitate matching of treatment philosophy with the patient's style. It may also be important to classify patients according to recovery style in investigations on the differential effects of treatment.

Serial No. M-AP(C)-17-1
1. Adult Psychiatry Branch
2. Section on Personality Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A Study of Problems in Growth and Adaptation in the Personality Development of the Adolescent

Previous Serial Number: Same

Principal Investigator: Roger L. Shapiro, M.D.

Other Investigators: Carmen Amor'os-Cabrera, M.A.S.W.; David Berkowitz, M.D.; Edward Shapiro, M.A., M.D.; John Zinner, M.D.

Man Years:

Total:	4.7
Professional:	3.2
Other:	1.5

Project Description:

Objectives: The overall pbjective of this study has continued to be the investigation of individual adolescent personality development in the context of his family and his social group. We have evolved a typology of families derived from observation of their interaction in conjoint family therapy. In particular, we have characterized families of adolescents with borderline symptomatology, narcissistic symptomatology, adolescents who do antisocial acting-out, and adolescents who are severely neurotic.

Methods Employed: Study of families of borderline adolescents, of narcissistic adolescents, of antisocial acting-out adolescents, and of neurotic adolescents has been done through observation of conjoint family therapy, marital therapy of the parents, and intensive individual therapy of the adolescent. Excerpts of family interaction containing defensive delineations of these adolescents by the parents provide evidence about the psychodynamic characteristics of the family and the unconscious assumptions of the family as a group. Data from the individual psychotherapy of the adolescent are the basis of formulations about the state of the ego in the adolescent, his capacity for object relations, and his level of defensive organization.

Major Findings: Comparison of families of borderline adolescents, narcissistic adolescents, antisocial acting-out adolescents, and neurotic adolescents reveals clear differences in the psychodynamic mechanisms determining defensive delineations of the adolescent by the parents. Parents of borderline adolescents manifest primitive splitting and projection of badness as the dominant

mechanisms underlying defensive delineations of the index adolescent. In our series this is true for at least one of the parents; this parent is the one most involved with the adolescent; the other parent may not manifest the same dynamic in delineation but is regularly acquiescent of the splitting and projecting parent and does not provide a corrective experience for the index adolescent. Unconscious assumptions dominated by splitting and projection are inferred in these families. Frequently these assumptions are fantasies intended to control expression of sexual or aggressive impulses which are seen as bad and destructive and which must be denied within the family. Frequently assumptions of compliance and sameness are required of family members, with the family boundary then containing what is good, and with the bad being projected outside the family. If the adolescent differentiates himself from other family members in his expression of impulses or ideas, he becomes bad and suffers feelings of extreme alienation from other family members.

Parents of narcissistic adolescents relate to their narcissistic offspring as narcissistic objects and use them to maintain their precarious self-esteem.

Parents of antisocial, acting-out adolescents are more differentiated than the parents of borderline adolescents. Projective mechanisms are the basis of defensive delineations, but the projections are less massive and are likely to be differentiated aspects of impulse life, like particular forms of sexual behavior (e.g., promiscuity, homosexuality) with more differentiated superego responses being retained within the parents. In addition to being more differentiated, the projections are more easily taken back by the parents during the course of family therapy. Unconscious assumptions are relatively differentiated fantasies which contain anxieties and concerns of the parents rising from their own internal conflicts, with one element in the conflict being projected into the developing adolescent. These unconscious fantasies differ from family to family but contain in them fantasies originating in one or both parents (e.g., fantasies of promiscuity, homosexual fantasies, etc.) which are projected by the parents into the adolescent. Covert gratification of these impulses in the parent or parents then occurs through their identification with the child.

Parents of neurotic adolescents employ predominantly regressive defenses rather than projective defensive. In their defensive delineations of the adolescent they remain oblivious to major areas of behavior in the adolescent which would otherwise be conflictful for the parents. Their behavior toward the adolescent is frequently dominated by repression of conflict at the Oedipal level in themselves, leading to repressive defenses in delineation of the adolescent in areas of sexuality, competition, and competence. Unconscious assumptions of the family as a group may be inferred which have differentiated and elaborated Oedipal themes.

Significance to Mental Health Research: As a typology of families of borderline, antisocial acting-out, and neurotic adolescents is articulated in this work, it is clear that it has a general importance beyond knowledge about the family behavior underlying disturbance in adolescence. This work suggests that it is important to understand emotional disturbance in the context of the family and group dynamics rather than to conceptualize disturbance as the fate of

an isolated, "sick" individual. This understanding of disturbance in an individual as a manifestation of disturbance in a family counters the tendency in families and in the community to scapegoat individuals by isolating them as sick and attempting to locate all disturbance in them. Consequently, this study has important implications both for the prevention of emotional disturbance and for family and community programs of treatment.

Proposed Course of Project: Current work with families of borderline, acting-out, and neurotic adolescents will be terminated on June 30, 1974.

Honors and Awards:

Roger L. Shapiro, M.D.: Director, Conference on Group Relations, sponsored by The A. K. Rice Institute of The Washington School of Psychiatry, Mt. Holyoke College, South Hadley, Massachusetts, June 7-16, 1974.

Publications:

Berkowitz, D., Shapiro, R., Zinner, J., and Shapiro, E.: Family contributions to narcissistic disturbances in adolescents. International Rev. Psycho-Anal., in press.

Berkowitz, D., Shapiro, R., Zinner, J., and Shapiro, E.: Concurrent family treatment of narcissistic disorders in adolescence. International J. Psychoanal. Psychother., accepted for publication.

Shapiro, R., Zinner, J., Berkowitz, D., and Shapiro, E.: The impact of group experiences on adolescent development. In Sugar, M. (ed.): The Adolescent in Group and Family Therapy. New York, Brunner/Mazel, in press.

Zinner, J.: The implication of projective identification for marital interaction. In Gruenbaum, H. and Christ, J. (eds.): Marriage: Prospect and Problems. Boston, Little-Brown, 1974.

Zinner, J. and Shapiro, E.: Splitting in families of borderline adolescents. Seminars in Psychiat., 6: 159-167, 1974.

Zinner, J. and Shapiro, R.: The family as a single psychic entity: Implications for acting-out in adolescence. International Rev. Psycho-Anal., accepted for publication.

Serial No. M-AP(C)-17-3
1. Adult Psychiatry Branch
2. Section on Personality Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Psychogalvanic Response in Family Therapy

Previous Serial Number: Same

Principal Investigator: John Zinner, M.D.

Other Investigators: None

Cooperating Units: None

Man Years: None

Project Description:

This project was terminated shortly after July 1973, at the time when the Principal Investigator left NIMH.

Serial No. M-AP(C)-17-4
1. Adult Psychiatry Branch
2. Section on Personality Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A Follow-up Study of 37 Families Treated in the Study of Adolescent Identity

Previous Serial Number: Same

Principal Investigators: Carmen Amor'os-Cabrera, M.A.S.W. and Roger L. Shapiro, M.D.

Other Investigator: David Berkowitz, M.D.

Cooperating Units: None

Man Years:

Total:	1.20
Professional:	1.15
Other:	0.05

Project Description:

Objectives: To obtain information about the current adjustment of the adolescent and his family as related to (1) the problems that motivated their admission to the project, and (2) their individual perceptions of the effects of therapy to any changes that may have taken place.

Methods Employed: The family group, the adolescent, and the parents were seen in separate interviews with the goal of providing information which would allow for the isolation and identification of various factors comprising the therapeutic experience, such as the impact of the adolescent's hospitalization, ward milieu, family therapy, couples therapy, and individual therapy.

Major Findings: While adolescence and adolescent turmoil are frequently seen as an opportunity for the growth and development of the child, little attention has been given to the possibility of change in the parent(s) during the child's adolescence. The findings of our study indicate that parents, precipitated into couple's psychotherapy by the adolescent turmoil of their children, have considerable capacity for psychic change at that time. We distinguished three stages of regression and progression through which these couples pass during the therapeutic process: (1) loosening of ties to the original family, (2) renewed adolescence and early marriage, and (3) return to parenting.

Significance to Mental Health Research: This study was useful in isolating factors contributing to the precipitation of serious adjustment problems during adolescence - for both adolescents and their parents - and in evaluating outcome of a treatment program derived from research findings.

Proposed Course of Project: The project will be terminated as of June 30, 1974.

Honors and Awards: None

Publications: None

Serial No. M-AP(C)-17-5
1. Adult Psychiatry Branch
2. Section on Personality Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cognition and Identity Development in Early and Late Adolescence: A Longitudinal Study

Previous Serial Number: Same

Principal Investigators: Stuart Hauser, M.D. and Roger Shapiro, M.D.

Other Investigators: None

Man Years:

Total:	0.30
Professional:	0.20
Other:	0.10

Project Description:

Objectives: The Principal Investigators have completed the investigation of cognition and identity formation in early and late adolescents of both sexes who are of patient and nonpatient status. Several contrasts of interest were built into the design: patients versus nonpatients; early versus late adolescents; male versus female adolescents; and each of the main groupings (patients and non-atients) were subdivided in terms of sex and age for intragroup comparisons, as well. All of these subjects were studied over a two-year period.

Methods Employed: The instruments for studying the subjects included a self-image interview, a Q-sort, and a battery of cognitive tests. The Q-sort was a major technique of the study as it focused on self-images. Modifications of the Q-sort technique developed for this project made it possible to focus more intensively on dimensions of each self-image, as well as overall integration and changes in self-images; these were analyzed at single points in time and over spans of time.

Major Findings: The cognitive tests focused on processes of categorization, differentiation, conceptualization, and problem-solving. The cognitive data were analyzed in terms of subsample differences at single points of time and change over time. In addition, correlations and parallels between self-images results and cognitive results were done. Performances of normal and disturbed adolescents were contrasted in early and late adolescence.

Proposed Course of Project: The project has been terminated.

Honors and Awards: None

Publications:

Hauser, S. and Shapiro, R.: Differentiation of adolescent self-image. Arch. gen. Psychiat., 29: 63-69, 1973.

Serial No. M-AP(C)-18-2
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: Coordinating "Micro-Codes" in Family Consensual Experience: A Study of the Responses to Speech Hesitancy and Fluency in Family Interaction.

Previous Serial Number: M-AP(C)-18-2

Principal Investigator: David Reiss, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.4
Professional:	.1
Other:	.3

Project Description:

Objective: The card-sorting experiment and previous studies by Reiss as well as by Strodtbeck, Mishler, and Waxler, et al., support the notion that families can be distinguished by the shared experience, of their members, of their environment. Furthermore, it appears as if this shared experience serves as a major regulator of moment-by-moment family interaction in a variety of settings. In order to maintain such consensual experience and to utilize it to deal with environment events as they occur on a moment-by-moment basis, family members must be exquisitely sensitive to each other's ideas and feelings at all times. The project was part of a card-sorting experiment in which family members could communicate only by voice; thus each member had to utilize the other's vocalizations to remain continuously integrated in this delicately balanced family process. Recent experimental evidence suggests that family members may utilize changes in each other's patterns of speech hesitancy and fluency for this purpose. We postulated that all families will show significant moment-by-moment response to changes in the fluency patterns of each member. However, some families--which we have termed "environment-sensitive"--are predicted to respond to hesitant speech as a signal pointing to a difficulty or incongruity perceived by the speaker in the laboratory puzzle itself. Other families--we call them "consensus-sensitive"--will interpret hesitancy as a sign of the speaker's perception of or response to the family itself. These

family types are defined by a consensual experience model and identified by their non-verbal performance on a card-sorting procedure. Thus, this study is a further test of the consensual experience model which predicts that family members will respond to minimal changes in each other's speech in ways determined by their over-all shared experience of the environment. These minimal speech changes could then be viewed as a personal, family "micro-code" serving to regulate its interaction in moment-by-moment harmony with its shared experiences and views.

Method: The verbal interaction in the second family problem of the card-sorting task was recorded on a specially prepared 4-channel tape recording system and transcribed by polygraph to give a visual, objective record of patterns of vocalization. A timing signal permitted coordination of the polygraph record with a typescript of the content of the family's discussion. Changes in fluency were measured by methods of Boomer and Dittman, and Goldman-Eisler, using the polygraph record. The family's response to fluency changes in any member was measured using interaction codes developed by Reiss and Mishler and Waxler, using the typescript, and also measures of the card sort and trial times.

Progress: Data collection and coding is complete. Preliminary data analysis shows that there is sufficient variability of fluency scores and interaction code scores to perform the necessary analyses. Programs are currently being written to measure the relationships between speech fluency on the one hand and acknowledgment and focus on the task on the other; these programs will use T, the information transmission statistic.

Serial No. M-AP(C)-18-3
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: The Effects of Progressive Isolation of an Individual From His Family on His Perceptual Functioning: Use of a Teletype-LINC Apparatus to Study the Reciprocal Relationship of Family Interaction and Individual Thinking.

Previous Serial Number: M-AP(C)-18-3

Principal Investigator: David Reiss, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.1
Professional:	.0
Other:	.1

Project Description:

Objective: Previous experiments in this Section have demonstrated that family interaction does have a specific, predictably short-term effect on the thinking and perception of its members. A critical question is: of all the flux and nuance of family interaction, which aspects are the most critical for regulating the thought and perception of its members. In the card-sorting and micro-code experiments, Dr. Reiss explored the role of various details of the vocalized portion of family interaction--excluding all forms of non-vocal performance. In the current experiment, the question is: to what extent do families differ in the amount of objective information--concerning the problem solution--they can obtain and distribute among its members; and, further, how does this obtained and distributed information regulate perceptual processes in their members. In other words, in what way does an individual's perceptual process--occurring in the midst of a family problem-solving task--depend on the quality and quantity of information provided him about the problem by his family, disregarding all other aspects of the family interaction of which he is a part. It is predicted that families will differ in the amount of relevant information they can discover about the problem, their effectiveness in distributing it to all members of the family and the use each member makes of information provided to him by others in the family. Specifically, we expect

families we have designated as environment-sensitive will extract a great deal of problem-relevant information from their environment, will effectively distribute it amongst members in the family and each member will utilize the information insofar as it assists him towards a logical, consistent and comprehensive problem solution. Individuals in families designated as consensus-sensitive will work to obtain, distribute, and utilize problem-relevant information only insofar as it permits a family solution at a desired level of within-member agreement. In the present experiment the procedure progressively isolates each member from his family. In the first condition all information obtained by all members about the problem is transmitted immediately to each individual along with the identity of the family member who obtained it--the "public mode"; in the second condition the information is transmitted after a variable delay without the identity of the member who obtained it--the "anonymous mode"; in the third condition no information is transmitted between members--the only information available to each member is what he obtains himself and that provided by an impersonal, extra-family source--the "standard mode." It is predicted that members in environment-sensitive families will maximally utilize information from any source whereas members in consensus-sensitives will utilize information from their past when it comes from an impersonal source. In order to provide immediate and standardized information transmission between the family members and between the family and the impersonal environment a teletype-LINC apparatus was constructed and programmed. This permitted the conduct of experiments entirely under the control of computer. It further permitted every item of family interaction, i.e. transmission of information by use of teletypes, to be precisely recorded in time. Thus, at the conclusion of the experiment it is possible to know not only what interaction has occurred but precisely when. This will permit an exact correlation of the various events comprising the flux of family interaction in this experimental setting. The teletype-LINC apparatus represents a major innovation in experimental social psychology and this project, in addition to its exploration of a particular hypothesis about family interaction, serves as an initial study of this innovation.

Method: Four family members, the parents and two children, are seated in booths so they cannot see each other. Each has a teletype and is instructed in its use. They are told that they must discover a class of symbol sequences that, when typed, will result in plus (+) being typed in return--automatically. Their understanding is that the plusses are typed in a systematic way and their job is to discover the system or pattern. Each member is given an example (e.g. CSTTTTTTTS which represents the class of "plus sequences" beginning with CS followed by n number of Ts and concluding with an S). Then each member tries out his own sequences in turn. In the "public mode" each sequence is automatically typed, immediately, on the other members' teletypes, with a symbol indicating whose sequences it was and whether it received a plus or not. In the "anonymous mode" the sequence is typed, along with its plus or minus, after a variable delay in a way concealing its authorship. In the "standard mode" the sequence typed by each member is not typed on anyone else's teletype; instead, a sequence from a standard library, stored in the computer, is typed on everyone else's teletype. Each mode is introduced by detailed instructions

and considerable practical experience for the family so that they become fully familiar with its distinctive features before beginning the actual problem-solving. Preceding and following each experimental problem each member is given an objective test of his ability to predict the class of sequences that will get a plus; this serves as a test of his ability to recognize relevant patterns.

Progress: In the first study, four families designated as consensus-sensitive were compared with four environment-sensitive families. The distinction between these two groups was made by the use of the card-sorting procedure. The consensus-sensitive families were those that sought to achieve consensus at the price of accurate problem solution. The two groups were carefully matched for a number of variables including intelligence and social class. The results were very striking and clear even though, in this pilot study, the sample was small. We used a matched-pair design in which an environment-sensitive family was matched with a consensus-sensitive family. In each pair the consensus-sensitive family showed a progressive improvement in problem-solving ability as intermember access was reduced from the public, to the anonymous to the standard mode. In sharp contrast each environment-sensitive family showed no change or a slight decline in problem-solving ability as intermember access was reduced.

A second study, using a wider sample of 15 families, studied the more detailed processes of hypothesis testing in the two groups of families. Correlational studies suggested that members in families with a marked deficit in the public mode relative to the standard mode, used others' hypotheses as a basis for their own hypothesis testing less often than members in families without such a relative deficit. Moreover, in the public deficit, members were less willing to risk the construction of incorrest hypotheses. These findings were predicted by a consensual experience model: it states that members in environment-sensitive families (with no public deficit) use each others' experiences with the environment as a way of augmenting their own experience and understanding of it, whereas consensus-sensitive families, since they are oriented to achieving an agreed-upon internal version of experience, tend to ignore the others' interaction with the environment. The unwillingness of these individuals to risk negative feedback, for their hypotheses, confirms our view that they experience negative feedback as censorious rather than information-giving; this attitude is consistent with their view of the environment as hostile and unknowable.

After completing the two studies described above, the program for the teletype experiment was substantially revised to: (a) make the three modes more nearly identical in the type and quantity of feedback for subjects' hypotheses and (b) to more completely automate the procedure; this now includes some automatic instruction and testing of subjects. Programming on the SEL has continued throughout the year and is now complete. Several pilot runs have been collected; systematic data collection will be completed in the coming year.

Serial No. M-AP(C)-18-6
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: Nurse-Doctor-Patient Interaction: An Experimental Study of Its Role in Patient Acculturation on Psychiatric Wards.

Previous Serial Number: M-AP(C)-18-6

Principal Investigator: David Reiss, M.D.

Other Investigators: None

Cooperating Units: Richard Almond, M.D., Department of Psychiatry, Stanford University Medical School, Palo Alto, California

Man Years:

Total:	.1
Professional:	.1
Other:	.0

Project Description:

Objectives: The major objective of the study is the direct measurement, under controlled conditions, of nurse-doctor-patient interaction as it occurs on psychiatric wards. The hypotheses of the study are derived from previous work by Dr. Almond which suggest that particular forms of nurse-doctor-patient interaction are crucial to inducing change and improvement in patients. Moreover, his previous studies suggest that certain value orientations by the staff serve to control or perpetuate these crucial interactions and many patients, after modifying their behavior during interaction with professional staff (and other patients), accept these values. This study seeks to relate value orientations of the staff, concerning types and process of treatment as well as personal preferences for types of interaction in group involvement, with the way they interact with each other and with patients. Specifically, we attempt to predict how a nurse and doctor will interact with each other from a knowledge of their value orientations; then we seek to predict how the nurse-doctor dyad will interact with a patient, based on their behavior when interacting as a dyad. This is the first direct experimental study of interpersonal processes amongst psychiatric personnel and therefore the project also serves the general purpose of examining the value of experimental techniques in this area of clinical investigation.

Method: Dr. Almond has modified a questionnaire he used in his original studies in New Haven. It has a broader range of items and is now suitable for administration to nurses and doctors as well as patients. The results of this questionnaire were used to evaluate nurses' and doctors' value orientations. These orientations, in turn, were used to predict nurse-doctor interaction. Two value orientations were selected as independent variables: 1) the subjects' expressed valuation of social openness and involvement in the ward (SOWI) by patients and staff; 2) the subjects' expressed wish to be included in the control groups (FIRO). Sixteen nurse-doctor dyads were formed so that they could be divided into four subsamples of equal size; 8 dyads were highly discrepant on SOWI (more higher) and 8 had similar scores; half of each of these subsamples had highly discrepant FIRO scores (more higher) and half were the same. Each dyad was tested separately in a small-group-type laboratory where they were isolated in booths and could communicate only by microphone. They worked on a standard set of problems, provided by the experimenter, requiring them to group a set of fictitious patients into activity groups. After a series of such problems, they were joined by a patient and continued to work on a set of similar problems as a triad. A number of objective indices of social interaction in the dyads and triads were derived from the card placements and objectively recorded times of various phases of the experimental task. These variables sought to measure the extent to which the dyads and triads 1) agreed or disagreed on problem solutions; 2) blindly followed a potent leader or worked interdependently with each individual making a substantial contribution; 3) utilized the full range of ideas elaborated by each person working alone; 4) "objectified or dimensionalized" the problem rather than seeing each fictitious patient and grouping as unique and personal.

Progress: Data analysis and written reports are complete. The basic findings are these: 1) There is a substantial difference in the effects of independent variables early in the task, when the nurse and doctor are being acquainted and where the dyad meets the patient for the first time, and later in the task when all three have been working together. 2) Where the nurse has a tendency to dominate groups and the doctor wants to be dominated (FIRO compatibility) and the nurse has higher SOWI values, the doctor and nurse form a very tight consensus but are unable to include or assimilate the patient. Here the dominant nurse shows evidence of using her more differentiated values as a club forcing the doctor to accept her views. These findings were also obtained where the nurse and doctor are equal in values and neither is dominating or wishes to be dominated (FIRO incompatible). Here the lack of any differences between the two on value orientation appears to produce an enui exaggerated by incompatibility and papered over by an artificial consensus. 3) Where the nurse and doctor are equal in values and FIRO compatible, and where the nurse is superior in values and the dyad is FIRO incompatible, the nurse and doctor form a flexible and varying consensus and very effectively include the patient in this consensus. In the former, interpersonal compatibility enables the dyad to reach an affable agreement which is well able to include a third person. In the latter, some interpersonal incompatibility focuses the dyad away from the task of simply gratifying one another. Rather,

they concentrate on preparing for the patient's entrance. The nurse's high SOWI values enable her to take an important role in assimilating the patient when he does arrive. The findings indicate that both independent variables are important and have an interacting effect. A difference in values appears crucial for stimulating the nurse-doctor dyad to consider various approaches and reach a meaningful consensus; however, if the nurse tends to dominate the dyad and the doctor acquiesces, the nurse will use her distinctive value position as a club. Tension between nurse and doctor will be papered over with a forced consensus and the patient will be excluded.

Further data analysis of individual triads during the past year revealed three additional mechanisms responsible for successful assimilation of the patient. First, the staff dyad is able to work out--in advance of the patient's arrival--a clear-cut principle for approaching the task. Second, both staff members share a belief in the strength of group process, i.e. that groups have a mutative or healing power. Third, the staff members must be able to set aside or ignore the test-like aspects of the experimental situation and take a lively and intense interest in the patient assignment task itself.

Publications:

1. Reiss, D., Almond, R., and Shephard, R. "Assimilating the Patient Stranger. Experimental Studies of Psychiatrist, Nurse, and Patient Interaction." J. Nerv. Ment. Dis., 158: 118-141, 1974.

2. Reiss, D., "Assimilating the Patient Stranger: Experimental Case Studies of Conflict Resolution Between Psychiatrists and Nurses." Psychiatry, in press.

3. Reiss, D., "Competing hypotheses and warring factors: Applying knowledge of schizophrenia." Schizophrenia Bulletin, 8: 7-11, Spring 1974.

Serial No. M-AP(C)-18-10
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: The Effect of Stimulus Materials on Family Problem Solving.

Previous Serial Number: M-AP(C)-18-10

Principal Investigator: David Reiss, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.1
Professional:	.0
Other:	.1

Project Description:

Objective: In the work done in our laboratory almost all family problem-solving tasks have utilized pattern recognition tasks in which patterned stimuli have been generated by one or another type of finite state grammar (as originally described by Chomsky and Miller). These problem materials have a number of advantages for studying complex perceptual and cognitive processes. Nonetheless, they represent a restricted model of the types of stimulus experience families may encounter in their everyday lives. It is important to establish that our findings concerning family problem solving can be generalized to different kinds of stimuli. Other features of the card-sorting procedure, such as the partial self-administration features, ease of observer recording of sorting performance, uniformity of physical characteristics of the stimulus materials, isolation in booths, have solved a number of long-standing methodological problems. Thus, it seemed inprudent to change the basic card-sorting procedure itself. However, a change of the content of the cards seemed useful to test the generality of our findings.

Method: Accordingly, we designed a procedure, adapted from Tien's Organic Integrity Test, which required subjects to sort cards according to objects pictured on the cards. Three sets of 15 cards were developed: two were used for initial and final individual task and a third for a family task between the individual tasks. Thus, the procedure has the same formal structure as the

letter syllable and number-sorting tasks. In each of three tasks the cards can be grouped in at least three ways: by color of object, use of object (e.g. tool, wearing apparel or sporting equipment) or by sex of user (male, female or neutral). A maximally effective sort would presumably use one of these three with complete consistency (corresponding to recognizing the finite state grammar in the basic sorting procedure). Also, families could vary in the amount of sorting similarity between members and across trials. Therefore, the same variables of problem-solving ability, coordination and closure were obtainable from this procedure as the basic procedure.

Progress: Pilot testing completed and the project was discontinued.

Serial No. M-AP(C)-18-12
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: Family Views of Its Social Environment: Effects on Family Therapy Process.

Previous Serial Number: M-AP(C)-18-12

Principal Investigator: David Reiss, M.D.

Other Investigators: Ronald Costell, M.D. and Loann Drake

Cooperating Units: Section on Personality Development, APB, IR, NIMH and Psychiatric Institute of the District of Columbia

Man Years:

Total:	2.9
Professional:	.5
Other:	2.4

Project Description:

Objective: During the past 6 years a series of laboratory studies have revealed that families--functioning as a group--develop shared and vivid concepts of the structure of the larger social community in which they live and work. Some families see their social environment as being patterned and engrossing. Other families see their social environment as threatening, chaotic and overwhelming. Evidence suggests that these shared family constructions of their environment are typical and enduring and dominate a family's response to any novel or stressful challenge in their social environment. We hypothesize that this is particularly true when a family must involve itself in an entirely new social community. Then, their typical reaction patterns play a major role in shaping their adaptations to and involvement in the institution's social processes. This kind of encounter between family and the new institution is very well exemplified by a psychiatric hospital that emphasizes family treatment. When the index member is admitted to the hospital, the whole family is actively engaged in the treatment program. Thus, the family--as a group--must orient itself to a set of novel and stressful challenges. This study is designed to test the relationship between family reaction patterns, as measured in the laboratory, and their adaptation during the first six weeks of family-oriented in-patient psychiatric treatment. The laboratory measures will be used to predict the degree to which the family will become involved and

engrossed in the treatment program or withdraw and protect itself from the hospital community and its programs. The interaction between family and hospital will also be viewed as protypical of interactions between families and a variety of institutions in their social environment. Later, we plan studies of the interaction between families and schools and families and occupational settings.

Method: Our independent variable is the family's problem-solving behavior in the laboratory. Based on this, a family can be placed in one of three categories: environment-sensitive, consensus-sensitive and distance-sensitive.

The dependent variables are selected to measure various aspects of the family's adaptation to the hospital community and its treatment program. Two procedures estimate the family's shared experience of the ward.

One procedure is the Ward Perception Q-Sort. Our theory states that a family's typical approach to construing novel social situations will determine its shared experience of the ward community. This shared experience is a variable intervening between family type and adjustment to the treatment program. The Ward Perception Q-Sort is intended as a direct estimate of this shared perception in the family. Specifically, it measures four aspects of the family's shared perceptions: accuracy, stereotypy, coherence and the similarity between members. The procedure requires family members, each individual in his own booth, to sort a group of 36 cards into 7 categories. Each card contains a description that might be applicable to the psychiatric ward and the 7 categories are labeled in descending order from "most characteristic" to "least characteristic." The family is urged to discuss together, through an intercom, how to categorize the cards. Objective scores are computed to estimate the four aspects of family perception from their sorts.

A second procedure is the Family Perception Procedure. This procedure is aimed at assessing, from a different perspective, the character of the family's experience of the social community--in this sense supplementing the Ward Perception Q-Sort. Here, a family is tested immediately following a multiple-family group meeting. Members, working together, are asked to make a number of distinctions between different families and between different members of the same family. Following general procedures outlined by George Kelly, an estimate is made of the number, complexity and character of the dimensions the family uses to discriminate between individuals and families within the group. Here, we attempt to distinguish between simple, moralistic and superficial distinctions and complex, flexible and psychologically insightful distinctions.

Three other procedures permit a direct assessment of family-hospital interaction; they are utilized directly on the ward. Both depend on the family's participation in a multiple-family therapy group--a major treatment modality at both the Psychiatric Institute and NIMH.

The first procedure is direct observation and measurement of the family's interaction with other family and psychiatric staff in the group. Over a

series of sessions the family's seating arrangement and the frequency and direction of their speech will be carefully recorded by an observer. The family's engagement and involvement in the ward community will be indexed by its willingness to disperse itself physically in a group rather than stay huddled together in adjoining seats; it will also be indexed by frequent speech directed at many individuals.

The second procedure is the sociometric technique. It, too, is being used as a measure of family engagement and involvement. In particular, we are interested in the experiential boundary the family constructs between itself and the rest of the community. Family members are asked to choose others in the multiple family group whom they like and know well. Impermeable boundaries will be indicated by families who choose few others and where members choose others in the same family. Members in families with impermeable boundaries should also be the object to choice by few others, outside the family, in the multiple family group.

A third procedure is a group cohesiveness questionnaire adapted from studies of outcome and process in therapy groups of unrelated members. This is used to estimate the family's subjective sense of being involved in the multiple family group.

Overall, we have made the following predictions. Environment-sensitive families--who can develop progressively more complex, subtle and accurate solutions to laboratory problems--will show simple kinds of evaluations of the ward social community on the Q-Sort and Family Perception Procedure. They will also show evidence of greater engagement and involvement in the therapeutic process. Consensus-sensitive families--who force an early agreement among themselves on simple and stereotyped solutions to laboratory puzzles--will show similar characteristics in their conceptions of the social community on the ward and remain isolated and distant from the therapeutic program.

Progress: Data collection has been completed for the NIMH sample; since complete data collection was obtained on only 5 families before the in-patient unit was shut down, these data are regarded as useful though preliminary. The findings show: 1) interaction coding in the multiple family group can be done with very high reliability; 2) the Ward Perception and Family Perception tests can be administered without difficulty and are understandable to the families; 3) there are some distinct links between performance of the family in the laboratory and on the ward. On this third point, an example is the near-perfect relationship between the levels of intra-family communication in the laboratory and in the multiple family group.

Data collection from the Psychiatric Institute Sample has continued throughout the year. Complete data collection on 13 families has been completed; 30 families will comprise the sample. Scoring procedures for both the Family and Ward Perception tests have been developed and computer programs for scoring multiple family group behavior have been completed and applied to the first 13 families. Preliminary analyses here have shown that families behave

with remarkable consistency in the multiple family group setting. For example, families who participate at high rate continue to do so, in every meeting, over a period of several months; if the most actively participating member fails to attend, then someone else in the family will take up his role. It is this remarkable consistency of family behavior, vis-a-vis other families, that this study is designed to measure and analyze.

Honors and Awards.

1. Invited address, "Theory as Bootstrap," Theory Construction Workshop, Annual Meeting of National Council on Family Relations, Toronto, Ontario, October 16, 1973.

2. Invited address, "Experimental Studies of Family Interaction," St. Elizabeth's Hospital, January 11, 1974.

3. Invited address, "Family Views Its World: Experimental Studies," University of Rochester Medical School, March 19, 1974.

4. Invited address, "Experimental Studies of Family Membership Experience," George Washington University Department of Psychology, April 3, 1974.

5. Invited address, "The Family as Regulator of Patient-Hospital Interaction," George Washington University Department of Medicine, May 28, 1974.

Serial No. M-AP(C)-18-13
1. Adult Psychiatry Branch
2. Section on Experimental Group and Family Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: Values and Atmosphere on a Psychiatric Ward: Basic Dimensions and Institution Comparisons.

Previous Serial Number: M-AP(C)-18-13

Principal Investigator: Ronald Costell, M.D.

Other Investigators: David Reiss, M.D.

Cooperating Units: NIH Nursing Administration, Nursing Units 3 West, 3 East, 4 West, 4 East, and Psychiatric Institute of the District of Columbia

Man Years:

Total:	.3
Professional:	.2
Other:	.1

Project Description:

Objective: Studies of psychiatric wards indicate that the attitudes and values of staff and patients with regard to therapy-related behaviors play an important role in defining the therapeutic milieu and the nature of therapeutic transactions between patients and treatment staff. This study seeks to explore the relation between therapeutic milieu attitudes and values and aspects of individual disposition toward interpersonal relations. In addition, the study seeks to explore the relation between these two domains and the ways in which individuals perceive the ward atmosphere. These relations are explored through analysis of a composite paper and pencil instrument. Therapeutic milieu attitudes and values are represented on the questionnaire by several previously studied scales including the Almond Social Openness-Ward Involvement Scale, and the Therapeutic Ideology Scale of Strauss. Individual interpersonal disposition items are represented by the A-B Therapist Scale of Whitehorn and Betz, the FIRO-B scales of Schutz and the Machiavellianism Scale of Geist and Christe. The Ward Atmosphere Scale of Moos provides the measure of perception of the social atmosphere of the ward. Exploration of therapeutic milieu attitudes and values, and of interpersonal relations disposition in conjunction with another study, in 1969, provided interesting factor analytic factor clusters from two institutions. In the current study we plan to assess the reliability of these

factors over time and extend the perspective of the study to perceptions of ward atmosphere.

Method: The instrument was administered to inpatients, nursing staff and professional staff on the four psychiatric units at NIMH and to similar groups at a large private psychiatric hospital, the Psychiatric Institute of D.C. Individuals from both hospitals participated in the 1969 study. The data consisting of score values of the various component scales will be factor analyzed using the principal components method. Reliability of the questionnaire components and factor structure obtained in the 1969 study will also be assessed. The total sample of respondents will be about 250.

Progress: Data analysis has been completed and the major results included in scientific reports. The two sets of principal findings are these. First, the various scales measuring ward values can be reduced to three, conceptually pure dimensions. The FIRO affiliation and inclusion scales form a Personal Affiliation dimension; the Social Openness, Sociotherapy and Nurturance scales form a Trust in Groups dimension; and the Psychotherapy, Somatotherapy and Benign Autocracy scales form a Trust in Conventional Authority dimension. Each dimension shows some mix between personal values and the more objective preference for treatment method; this confirms the hypothesis that preferences for certain treatments are rooted both in empirical evidence and personal preference and this appears to be no more true for patients than for staff. The second major finding is that these dimensions hold remarkably constant across institutions (NIMH and Psychiatric Institute); across segments of the hospital population (staff and patients) and across years of administration (1969 and 1972). In other words, the correlations between treatment preferences and personal values is remarkably constant in different populations of subjects measured at different points in time. The formal properties of these dimensions also lend themselves to the careful study of the relationship between ward values and the family therapy process.

Serial No. M-AP(C) 19-1
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Comparative Studies of Discordant Siblings in Families of Schizophrenic, Juvenile Delinquent and Well-adjusted Young Adults

Previous Serial Number: same

Principal Investigator: William Pollin, M.D.

Other Investigators: none

Man Years: none

Proposed Course of Project: This project had been temporarily suspended and had been held in abeyance. It is now terminated.

Honors and Awards: none

Publications: none

Serial No. M-AP(C) 19-2
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies in the Development of Personality and Psychopathology in Identical Twins Discordant for Schizophrenia

Previous Serial Number: same

Principal Investigator: William Pollin, M.D. and Richard Wyatt, M.D.

Other Investigators: Robert Belmaker, M.D.

Cooperating Units:

A. NIMH:
Laboratory of Clinical Science
Laboratory of Clinical Psychopharmacology

Man Years (computed for the 12 month period)

Total:	3.5
Professional:	1.0
Other:	2.5

Project Description:

Objectives:

In identical twins discordant for schizophrenia, genetic, racial, ethnic, social class and a variety of other variables which have seemed to bear some relationship to the pathogenesis of this illness are constant for both twins. Therefore, in studying pairs in which one twin is schizophrenic and the other is not, there exists an unusually favorable situation within which to attempt to determine what life history events, family relationship patterns and non-genetic biologic factors play a role in the etiology of this illness, and in what manner. In addition, a new method of analyzing and comparing twin and control data has made it possible to define probable genetic factors which predispose to schizophrenia. The overall picture of schizophrenia pathogenesis which is then possible enables one to aim at a more rational approach to development of new treatment modalities.

Methods Employed:

Previous reports have described the intensive multidisciplinary inpatient work-up of a total of 25 families with identical twins admitted for evaluation to the Clinical Center. The methods used during the past year are the same as those used previously.

Patient Material:

See previous reports for details of the twin population. The 23 normal controls were volunteers who had never had a psychotic illness. The average age of the controls did not differ from that of the patients and bloods were drawn and processed under identical circumstances.

Major Findings:

In last year's Annual Report we described the finding that platelet MAO was low both in the schizophrenic twins of our series and in their healthy identical co-twins. In contrast, platelet N-methyltransferase was elevated only in the schizophrenic member of each twin pair. We interpreted this to mean that platelet MAO is a genetic marker for the predisposition to schizophrenia, whereas N-methyltransferase is part of or secondary to the illness process itself. During the immediate past year we used the discordant twin series to study two other enzymes possibly related to catecholamine metabolism or psychopathology. Dopamine-β-hydroxylase (DBH) was found to be highly correlated between twins ($r = .86$) but not different in the twins than in normal controls. This was done in collaboration with Dr. Lamprecht of the LCS. Creatinine phosphokinase (CPK) was found to be significantly correlated between twins ($r = .56$) but only two twins were elevated. This was done in collaboration with Dr. Herbert Meltzer of the University of Chicago. A major effort in our laboratory this past year has been to clarify the mechanism of the genetic role of MAO shown by the twin study. We have used such techniques as polyacrilamide gel electrophoresis of the enzyme protein. We completed a study of menstrual cycle-related variation in an attempt to define non-genetic contributions to variations in level of platelet MAO, and found a 23% peak to trough effect of the menstrual cycle on enzyme activity. We have also been interested in whether the platelet MAO finding is meaningful only in the presence of severe psychopathology or whether platelet MAO and other catecholamine-related enzymes show relationships to normal personality variations. In collaboration with Drs. Murphy, Buchsbaum, Ciaranello and others we have studied a series of 100 normal volunteers with MMPI's, platelet and plasma MAO, and plasma DBH. Data is still being analyzed. In collaboration with Drs. Goodwin and Murphy we are studying possible relationships between platelet MAO and probenecid-induced accumulations of monoamine metabolites in CSF.

Significance to Mental Health Research:

Previous analysis has focused on the significance of defining a) non-genetic constitutional contributions toward the pathogenesis of schizophrenia, and b) a characteristic pre-illness life course preceding schizophrenia. The findings of the past two years suggest some of the genetically determined biological factors which contribute to predisposition to this psychosis.

Proposed Course of Project:

Continuing analysis of multidisciplinary data with further efforts to trace central relationships are proposed. New biochemical techniques as they become available will be used, as well as further examination of the substrates and inheritance of the enzyme abnormalities for monoamine oxidase will be pursued. In collaboration with Drs. Carpenter and Hawk of the Section on Psychiatric Assessment, we are planning to test the recently reported eye-movement finding in schizophrenia in the discordant twin series to see if it is a genetic marker.

Honors and Awards: none

Publications:

Lamprecht, F., Wyatt, R. J., Belmaker, R., Murphy, D. L. and Pollin, W.: Plasma dopamine-beta-hydroxylase in identical twins discordant for schizophrenia. In Snyder, S. and Usdin, E. (eds.): New Frontiers in Catecholamine Research. New York, Pergamon Press, 1974.

Belmaker, R., Pollin, W., Wyatt, R. J., and Cohen, S.: A follow-up of monozygotic twins discordant for schizophrenia. Arch. Gen. Psychiat. 30: 219-222. 1974.

Wyatt, R. J., Saavedra, J. M., Belmaker, R., Cohen, S. and Pollin, W.: The dimethyltryptamine-forming enzyme in blood platelets: a study in monozygotic twins discordant for schizophrenia. Am. J. Psychiat. 130: 1359-1361, 1973.

Serial No. M-AP(C) 19-3
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH

Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Coronary Prone Behavior Pattern in the NHLI Type II Study

Previous Serial Number: none

Principal Investigator: Robert Belmaker, M.D.

Other Investigators: William Pollin, M.D.

Cooperating Units: NHLI Type II Coronary Intervention Study
NHLI, Molecular Diseases Branch
NHLI, Cardiology Branch
Dr. Ray Rosenman, Harold Brunn Institute for Cardiovascular Research, San Francisco, Calif.

Man Years (computed for the 12 month period)

Total:	.75
Professional:	.25
Other:	.5

Project Description:

Objectives:

Studies in various centers have suggested a strong role for behavior as a factor in the pathogenesis of coronary artery disease. Specifically, a coronary-prone behavior profile has been developed that consists of haste, impatience, time pressure, and tenseness. The National Heart and Lung Institute is engaged in a large-scale study of patients referred with hypercholesterolemia. Some of these patients are determined to have coronary artery disease and others are determined to be free of coronary artery disease. A sub-sample of those with coronary artery disease are being followed prospectively by the National Heart and Lung Institute and evaluated with arteriograms for progression of coronary artery disease. The objective of our study is to determine if there are any differences in behavior patterns between those patients who have hypercholesterolemia, but do not have coronary artery disease, and those patients who have both hypercholesterolemia and coronary artery disease. In addition, an objective of this study will be to determine if behavior pattern affects the progression of coronary artery disease as determined by arteriograms. Thus, our study will determine whether behavior pattern is an important risk factor for coronary artery disease even in those patients already predisposed with hypercholesterolemia.

Methods Employed:

The patients are referred from the Type II Coronary Intervention Project and are given a 15-minute standard interview by Dr. Belmaker. This interview is videotaped. In addition they are asked to complete the Adjective Check List and the Jenkins Activity Scale which have been shown to correlate with coronary-prone behavior pattern. Dr. Belmaker is, of course, blind as to whether the patient has coronary artery disease or not.

Patient Material:

See above

Major Findings:

Eighty-four patients were interviewed and rated for coronary artery disease and Type A behavior pattern. No relationship was observed. Thus, it would seem that in a population metabolically predisposed to coronary disease, behavior pattern is less important than in the population at large. This is consistent with a multifactorial approach to this illness.

Significance to Mental Health Research:

Much effort has been expended to try to unravel the causes of our present national epidemic of coronary artery disease. If life style and behavior pattern are contributing factors, we need to know this in order to reduce incidence of the disease.

Proposed Course of Project:

Thirty-four of the above 84 patients were admitted as patients in the NHLI prospective research project on Type II hyperlipidemia. This will be followed for new evidence of coronary disease. After two years we will re-analyze the data to see if Type A patients have faster progression of disease than Type B.

Honors and Awards

None

Publications

None

Serial No. M-AP(C) 19-5
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Progress Report
July 1, 1973 through June 30, 1974

Project Title: The Distribution and Concomitants of Schizophrenia, and Other Psychopathologies, in a Systematic Sample of 15,909 Twin Pairs

Previous Serial Number: same

Principal Investigator: William Pollin, M.D.

Other Investigators: none

Man Years: none

This project has been temporarily suspended and is being held in abeyance.

Honors and Awards: none

Publications: none

Serial No. M-AP(C) 19-6
1. Adult Psychiatry Branch
2. Section on Twin & Sibling Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Twin Intrapair-Comparative Technique in the Study of the Determinants of Early Personality Development

Previous Serial Number: same

Principal Investigators: Mrs. Eleanor Dibble and William Pollin, M.D.

Other Investigators: none

Cooperating Units:

Social Work Section, NIMH
Child Research Branch, NIMH (Nursery School), Dr. Charles Halverson and Gail Inoff (M-CR-24 (C))
Department of Psychiatry, Georgetown University Medical School, Washington, D.C., Drs. Martin Allen and Mary Riedy
Yale University Child Study Center, New Haven, Conn., Dr. Donald J. Cohen
Adelphi Child Study Center, Dr. Stanley Greenspan

Man Years (computed for the 12 month period)

Total:	.5
Professional:	.5
Other:	0

Project Description :

Objectives:

1. To develop methodologies for the study of the antecedents of personality in children, based especially on the comparative study of pairs of twins. To study differences between children in relation to a) non-genetic biological differences between the twins, i.e. differences in the congenital endowment of the children and early differences during the first weeks of life; b) parental personalities and behaviors, specifically, differences in relationship and perceptions of parents to Twin A versus Twin B; c) the impact of such differences in parent-child relationship, and of traumatic experiences such as illness or surgery, on the child; and d) the origins of, and differences between the children in relation to behavior problems.

2. To study the developmental roots of personality and family characteristics which have been related in our other studies on schizophrenia and psychopathology to the origins of serious mental illness in twins.

Methods Employed:

(1) Longitudinal Study. Families of ten sets of twins who had been referred by local obstetricians have been followed since the twin pregnancy was diagnosed. The children's current ages in this cohort range from 3 to 6 years. During the years the children and their families have been followed, a protocol of testing has been accomplished: developmental evaluation and Bayley testing; neurological evaluation; psychiatric diagnostic evaluations of the twins and parents; periodic family interviews; information from the Experimental Nursery School of the NIMH in which the children were studied for a half-day at age 3; evoked cortical potentials; projective psychological testing; and social work evaluations. Dr. Martin Allen has interviewed the parents and Dr. Stanley Greenspan, child psychiatrist, interviewed the children at age five to six years to study the increasingly complex structuralization of their behavior as they develop more complex coping and defense systems and the fantasies. Special psychological testing including: a Rorschach test, a specially devised twin TAT, the "World Test" for children, the Stanford-Binet (IQ) test and the Bender-Gestalt test in order to gain information about the twins' psychic development (both conscious and unconscious).

(2) Epidemiological Study. On the basis of our studies on psychopathology and on the longitudinal study of normal twin development data, the study titled "Childhood Personality Development: Twins and the Antecedents of Personality," approved by the Technical Review Committee on March 31, 1972, was completed. This epidemiological investigation aims at exploring the hypotheses and conclusions from the previous studies and extends the size of the sample, explicates and operationally defines particular variables that have emerged from clinical observations, and uses newly devised psychological instruments. These instruments have been described elsewhere (1972 Annual Report).

The sample includes 425 families recruited to participate in this study by personal appearance at local Mothers of Twins Clubs and at the Annual Convention of the National Organization of Mothers of Twins Clubs.

Patient Material:

The Longitudinal Study consists of a cohort of 10 sets of twins, 8 monozygotic and 2 dizygotic. The cohort of the epidemiological study consists of 425 sets of twins.

Major Findings:

A. Longitudinal Study: During fiscal year 1974 the following has been accomplished: 1) all parents completed a newly devised form, Behavior Problems, on the children for a retest; and 2) five sets of children have been seen and tested according to the new procedure described above.

B. Epidemiological Study: During fiscal year 1974 the collection of data from 425 families is now in the process of analysis. Early analysis indicates the two newly devised scales, the Childhood Personality Scale and the Parents Report, are reliable in picking up subtle behavior differences between the twins and to reflect differences in the perceptions of fathers and mothers of their own behavior with a particular child. The remaining five questionnaires of the set still need to be tested, but indications are that the study will make a real contribution in relating child behavior to child rearing behaviors of parents thus adding to knowledge of personality development or where differences in development arise.

Responsibility for the work on the Epidemiological Study is carried jointly with Dr. Donald Cohen, Yale University Child Study Center. Future reports will give details of the analyses.

Both the Longitudinal Study and the Epidemiological Study of twins will provide a testing ground for our hypotheses about genetic and non-genetic antecedents of personality. The psychological instruments which have been developed for the epidemiological study will have broad applicability in other studies which aim at understanding the social and emotional development of children during the first years of life.

Proposed Course of the Study:

See above.

Significance to Mental Health Research:

The Longitudinal Study children are now entering the Oedipal and latency stages of development and offer the opportunity to observe the increasingly complex structuralization of their behavior as they develop more complex coping and defense systems. The Epidemiological Study provides a major testing ground of many of our hypotheses about genetic and non-genetic antecedents of personality, with particular relation to our interest in the first months of life, family perception and the development of competence. The questionnaires developed for this study should have broad application in other studies which aim at understanding the social and emotional development of children during the first years of life.

Honors and Awards: none

Publications:

Cohen, D. J., Dibble, E., Grawe, J. M. and Pollin, W.: Separating identical from fraternal twins. Arch. Gen. Psychiat. 29, 465-469, 1973.

Serial No. M-AP(C)-20-5
1. Adult Psychiatry Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychological Studies of Patients with Affective Disorders

Previous Serial Number: Same

Principal Investigators: Edward F. Donnelly, Ph.D., Dennis L. Murphy, M.D.

Other Investigators: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To study differences in psychological test response patterns between patients with affective disturbances and other psychiatric patients, and to explore the relationship between test response patterns and a range of clinical and experimental variables among patients with affective disturbances. A major goal is to clarify distinctions between patients with unipolar and bipolar affective disturbances.

Methods: Psychological test productions of patients with a variety of affective disturbances constitute the basic data for differentiation of subgroups. The test data include both objective and projective personality tests, as well as an intelligence scale. Patterns of psychological test data are studied in relation to aspects of premorbid adjustment, course of illness during hospitalization, and adjustment following hospitalization.

Major Findings: Differences have been observed in the test response patterns of patients with unipolar depressions, on one hand, and bipolar patients - whether depressed or manic - on the other. On the Rorschach test, patients with unipolar depressions develop responses which are dynamically expressive communications, while bipolar patients develop responses which are concretely related to the inkblots. During the past year, it has been established that among patients hospitalized for depression, a group of unipolar patients can be differentiated from a group of bipolar patients on the basis of patterns of responses to the MMPI. Moreover, there is a difference between unipolar and bipolar depressed patients in the relationship between scores on a depression scale of the MMPI and ratings for depression on the Bunney-Hamburg scales.

While there was a significant relationship between the two for unipolar patients, there is not for bipolar patients. This would suggest that there may be a difference between the subjective experience of depression and outward manifestations of depression in behavior among bipolar patients. See Individual Project Report M-CS-PS(C)-23 for findings in drug response differences in bipolar and unipolar depressed patients as related to personality characteristics.

Significance to Mental Health Research: The research may contribute to a refined classification of patients with affective disturbances related to psychological test performances, patterns of perceptual and cognitive functioning, aspects of premorbid functioning, and post-morbid adjustment.

Proposed Course of Project: Further analyses will be done on data at hand, and additional data will be accumulated on new patients admitted to the Clinical Center for the treatment of depression. All further data collection, analysis, and reporting will be incorporated in Individual Project Report M-CS-PS(C)-23, as Dr. Donnelly has transferred to another agency.

Honors and Awards: None

Publications: See Individual Project Report M-CS-PS(C)-23.

Serial No. M-AP(C)-20-6
1. Adult Psychiatry Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychological Deficits in Selected Neurological Disorders

Previous Serial No.: Same

Principal Investigator: Edward F. Donnelly, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.00
Professional:	0.00
Other:	0.00

Project Description:

Objectives: To study patterns of deficit in personality, intellectual, cognitive, and perceptual functioning in various neurological and psychiatric disorders, and to study drug-related changes in patterns of deficit.

Methods: Patients with various neurological disorders such as Parkinson's disease, Huntington's chorea, and Down's syndrome are studied with psychological measures of intellectual, personality, and central nervous system functioning. These include the Halstead-Reitan Battery, the MMPI, and the WAIS. Behavioral observations are recorded on the Bunney-Hamburg scale.

Major Findings: A study of the possible effects of L-DOPA on intellectual and memory functioning reveals that IQ increments reported by prior studies can be explained as a function of practice effect, a variable difficult to control in longitudinal studies requiring testing and retesting. A long-term follow-up of patients on L-DOPA therapy suggests that there are no deleterious effects on intellectual functioning, although there is a slight (and non-significant) decline in the scores on the Comprehension subtest of the WAIS for a portion of the patients.

Significance to Mental Health Research: From both clinically and empirically derived data, there have been claims for the improvement in intellectual functioning attributed to treatment with L-DOPA. Observations would suggest, however, that the improvement can more likely be attributed to practice effect on the tests than to L-DOPA induced effects.

Proposed Course of Project: The Project was terminated shortly after the start of the new Fiscal Year as the Principal Investigator transferred to another facility.

Honors and Awards: None

Publications: None

Serial No. M-AP(C) - 21-2
1. Adult Psychiatry Branch
2. Section on Perceptual and Cognitive Studies
3. Bethesda, Maryland

PHS - ADAMHA - NIMH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: Studies of Perceptual and Cognitive "Styles" in Psychiatric and Non-Psychiatric Subjects.

Previous Serial Number: M-AP(C) - 21-2, 21-4

Principal Investigator: Monte Buchsbaum, M.D.

Other Investigators: William P. Carpenter, Jr., M.D.; J. Christian Gillin, M.D.; Frederick Goodwin, M.D.; Lawrence Greenhill, M.D.; Dennis Murphy; M.D.; and Theodore P. Zahn, Ph.D.

Cooperating Units: Laboratory of Psychology; Laboratory of Clinical Science; and Laboratory of Psychopharmacology, NIMH.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives: To relate individual differences in sensory perception and cognitive functioning in human subjects to psychiatric clinical dimensions and to neurophysiological measures; to develop and implement clinically valuable computerized measures of perceptual and cognitive functions.

Methods Employed. Research in this section focuses on disturbances in perceptual and cognitive behavior in psychiatric patients. Its work is closely coordinated with basic electrophysiological and perceptual research in the Unit on Psychophysiology, Laboratory of Psychology, NIMH.

The finding of perceptual and attentional deficits in a variety of psychiatric patient groups has encouraged the development of theories of psychiatric illness and personality structure based on perceptual style. However, the psychiatric patient's report of his perceptions might reflect poor motivation, poor cooperation, failure to attend to instructions

or inability to communicate with the examiner, rather than any actual perceptual difference. For this reason the section has emphasized electrophysiological correlates of perceptual behavior using averaged evoked response techniques which demand relatively little active participation from the subject.

We have previously reported that bipolar affective disorder patients and acting-out adolescents manifested relatively greater rates of increase of visual average evoked response (AER) amplitude to increasing stimulus intensity (augmenting) than did unipolar, or nonparanoid schizophrenic patients, who often showed actual decreases in AER amplitude with increasing stimulus intensity (reducing).

Based on the results of several studies, we have viewed evoked response augmentation as indicative of a tendency to increase the perceived intensity of stimuli, to seek external stimulation, and to be relatively insensitive to extremely weak stimuli. Evoked response reducing, in contrast, is associated with a tendency to decrease the perceived intensity, to avoid exciting external stimulation and to be relatively sensitive to extremely weak stimuli. We have used two strategies to attempt to develop clinically significant diagnostic and prognostic evoked response tools. First, a series of clinical group comparisons to explore the range of variation and to further validate the concept: bipolar affective disorder patients actively incorporating sensory stimuli compared with schizophrenic withdrawal; hyperactive and acting out children compared with normal children and adults; narcotic addicts because opiates, which modify response to painful or intense stimulation, are selected by them as the drug of abuse. Second, we have done a series of neurophysiological studies in normal adult volunteers to attempt to understand the pertinent mechanisms which produce individual differences in stimulus-intensity response. These studies are reported under individual project report Serial No. M-P-C(C)-36, Laboratory of Psychology.

The clinical studies reported here all utilize a similar AER recording technique. AER to four intensities of light were recorded from vertex-right ear leads; components with a positive peak at about 100 msec (P100), a negative at 140 msec (N140) and a positive at 200 msec (P200) were measured.

Major Findings:

1. Affective Disorders. Several recent studies have demonstrated cortical average evoked response (AER) differences between patients with bipolar and unipolar affective disorders.

Using four intensities of visual stimuli, bipolar patients tend to show AER which increase with increasing stimulus intensity (augmenters) whereas unipolar patients tend to show AER which may decrease (reducers). These two groups of patients also differ in platelet monoamine oxidase (MAO) activity, with bipolars showing lower levels than unipolars. Within groups of affective disorder patients and some normal groups, AER amplitude intensity slopes and MAO levels are significantly correlated.

Both measures show differences between normal and patient groups, appear to be under genetic control (based on twin study evidence) and exhibit some relevant psychological test correlations in non-psychiatric patient groups. Both measures also show some differences in schizophrenia as well, with schizophrenics having both low AER slopes and low MAO activity. Individually, the AER and MAO measures separate between 55 to 68% of normal and patient populations correctly. However, a number of "normal volunteers" were found with MAO or slope levels below that of the lowest schizophrenic inpatient.

In order to further evaluate the possible diagnostic usefulness of these measures, a multivariate approach was utilized. Multivariate discriminate analyses were used with a group of bipolar, unipolar and schizophrenic patients and 49 normal controls with approximately the same age distribution. Since both the evoked response and the MAO have been found to interact with sex, discriminant analyses on males and females were run separately. In both sexes combined classification of the four categories was quite successful with over 72% of the subjects being classified correctly. Prominent in classification errors were bipolar individuals and normal individuals classified as schizophrenics.

In the female group the average evoked response measures were the most highly weighted in the discriminant analysis, having the first and second ranking weights for all four groups, with the MAO ranking third for the unipolar and normal groups. In the male subjects, MAO ranked first as a discriminator in bipolars, unipolars and schizophrenics and third as a discriminant in normals.

2. Schizophrenic Patients. Prognostic measures of patient improvement were studied in 16 unmedicated acute schizophrenics. The patient sample was divided into two groups, improved and unimproved, on the basis of global illness rating on the Nurses' Rating Scale by comparing the early (2 weeks) to the late (26 weeks) period of hospitalization. Eleven patients improved and five patients worsened or remained the same after 16 weeks.

Patients who were likely to improve tended to be reducers. All five patients who worsened or were unchanged were augmenters; only three of 11 patients who improved were augmenters.

3. D- and L-amphetamine Studies in Patients with Affective Disorders. A series of 11 patients have been studied on a double blind crossover study using oral D- and L-amphetamine. Both early (72-112 msec) and late (168-248 msec) AER components were studied. For late components previously associated with mood changes, D-amphetamine had a significantly greater elevating effect than L-amphetamine. For early components, associated with normal, schizophrenic and bipolar differences, L-amphetamine had a greater augmenting (amplitude-intensity slope increasing) effect; a marked rebound was seen with reducing 4 hours later.

4. Intravenous L-Dopa in Patients with Affective Disorders. Thirteen patients (7 bipolar and 6 unipolar were studied after being free from psychoactive drugs for 10 days. The drug MK-485 was begun 2 days prior to the study. Using a double-blind method of intravenous infusion, L-dopa 50 mg or placebo was administered; AERs were collected predrug, and at 2, 16 and 30 minutes. Within 30 minutes of the infusion of dopa, the AER showed an increase in the amplitude/intensity slopes for unipolar patients and a decrease for bipolar patients in early (72-112 msec) components.

Unipolar patients also showed a decrease in the amplitude of late (168-248 msec) components which have been found to decrease with oral dopa in man and IV dopa in monkeys. Bipolar patients did not show this effect, further distinguishing the two clinical groups. In contrast to the effects of dopa, the infusion of placebo was associated with changes in the slopes (168-248) which, while not of statistical significance, were in an opposite direction and suggested a procedure related stress effect.

Significance to Biomedical Research and the Program of the Institute:

This project, in helping to give a neurophysiological basis to psychiatric diagnosis and prognosis, contributes to the effort to understand the mechanisms of psychiatric disease.

Proposed Course of the Project:

1. Amphetamine-like compounds may briefly alleviate symptoms in some manic as well as some depressive individuals. Evidence has been reported that one might predict

anti-depressant efficacy of tricyclic drugs by the occurrence of a mood elevating effect of amphetamine. Pharmacological efficacy of lithium in affective disorders and of amphetamine in MBD children has been predicted by AER measures. Further, differential effects of oral D-L amphetamine have also been found with AER techniques. These suggestions that AER techniques may yield important predictors of individual differences in drug effect will be pursued by 1) expanding the clinical groups in the D- and L study to allow diagnostic category and mood elevation to be evaluated separately and 2) IV administration studies which allow rapid assessment of drug effect.

2. Longitudinal studies of bipolar patients. Daily AERs from bipolar patients are being obtained to observe the "switch" from mania to depression. A series of such individuals will be studied to 1) find AER predictors of the switch and 2) further clarify the trait/state relationship between early and late AER components. Correlations with biochemical and sleep records will also be obtained.

3. "Biochemical high risk" studies. The MAO-AER-psychiatric disorder links might be further clarified by examining the AER in a sample of "normal" individuals with extreme values of platelet MAO activity. Factors secondary to hospitalization and interactions between depression, hospitalization and low MAO activity would be diminished.

Individuals with low levels of MAO might be postulated to be at some increased high risk for development of bipolar depression or schizophrenia or subclinical symptons of either; use of the AER might separate these two categories. Further, examination of first degree relatives would yield valuable information about whether the conjunction of combinations of extreme values of MAO activity and AER yield additive, ameliorative or multiplicative effects.

Individuals with extreme platelet MAO levels will be located by sampling large numbers of student volunteers. AER, personality measures and a detailed family history will be taken.

Honors and Awards:

1. Consulting Editor, Physiological Psychology
2. Consulting Editor, Psychophysiology
3. Member, Program Committee, American Psychosomatic Society

Publications:

Greenhill, L. L., Rieder, R. O., Wender, P. H., Buchsbaum, M., and Zahn, T. P.: Lithium in the treatment of hyperactive children. Arch. Gen. Psychiat. 28: 636-640, 1973.

Buchsbaum, M. and Wender, P.: Average evoked responses in normal and minimally brain dysfunctioned children treated with amphetamine. Arch. Gen. Psychiat. 29: 764-770, 1973.

Buchsbaum, M., Landau, S., Murphy, D., and Goodwin, F.: Average evoked response in bipolar and unipolar affective disorders: Relationship to sex, age on onset and monoamine oxidase. Biol. Psychiat. 7: 199-212, 1973.

Serial No. M-AP(C) - 21-4
1. Adult Psychiatry Branch
2. Section on Perceptual and Cognitive Studies
3. Bethesda, Maryland

PHS - ADAMHA - NIMH
Individual Project Report
July 1, 1973 - June 30, 1974

Project Title: Perceptual and Cognitive Style in Normal Twins.

Previous Serial Number: M-AP(C) - 21-4

Principal Investigator: Monte Buchsbaum, M.D.

Other Investigators: Lyman C. Wynne, M.D., Ph.D.
Theodore P. Zahn, Ph.D.
Stephen Landau, M.D.

Cooperating Units: Unit on Psychophysiology, LP - NIMH

Project Description:

Objectives: Evaluation of the heritability and interaction of several sensory, perceptual and cognitive functions in normal volunteer twins.

This project is now incorporated into M-P-C-(C) - 36, Laboratory of Psychology.

Serial No. M-AP(C)-22-1
1. Adult Psychiatry Branch
2. 3-West Clinical Research Unit
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychological and biological interactions in manic-depressive and schizophrenic psychoses

Previous Serial Number: None

Principal Investigator: Robert M. Post

Other Investigators: John Carman, Frederick Stoddard

Collaborating Scientists: F.K. Goodwin, J. Christin Gillin, Monte Buchsbaum, Robert Belmaker, Michael Ebert, William E. Bunney, Jr.

Psychiatric Consultants: H.A. Meyersberg, J. Smith

Cooperating Units: Nursing personnel on 3-West and Occupational Therapist (Eleanor Stapin, 1/2 time), Social Work personnel (Robert Savard, Eleanor Dibble, Stan Hirsch), and Psychologist Betsy Sherwood

Man Years:

Total	5.7
Professional	4.2
Other	1.5

Project Description:

Objectives: The objective of this project is the development of a research program for the study of the interactions of behavioral, biological, and pharmacological processes in acute manic, depressive, schizophrenic, and drug related psychoses. Focus is given to separating primary or etiological biological changes from those that are secondary or epiphenomena of the behavioral changes under scrutiny.

The development of quantitative measures of clinical variables that are distinguishing features as well as thost that are common in the acute psychoses is continuing. Delineation subgroups of patients that respond to specific treatment strategies is sought, particularly the relationship between clinically and biochemically defined subgroups in predicting pharmacological response and prognosis of the illness. Study of central nervous system transmitter metabolism in psychotic and recovered states and the elucidation of the mechanism of action of psychoactive drug is a primary objective of the program. Corollary programs for study of biochemical-behavioral interactions

in laboratory animal "models" are being pursued.

Methods Employed:

1. Acutely psychotic patients with clear diagnoses of manic, depressive, or schizophrenic illness are selected for the program. Behavioral and biochemical data are collected on a longitudinal basis throughout the entire hospitalization during both drug-free and pharmacological treatment periods.

2. Behavioral Data and Psychotherapy.

a. Twice daily, a trained nursing team rates patients on global items of depression, mania, anxiety, psychosis, anger, as well as on a more detailed 34 item scale designed (by Dennis Murphy) to account for changes in both intensity and frequency of symptoms. Nurses and patients are "blind" to all pharmacological manipulations. Ratings are performed at 2 hour intervals for more acute studies. Observation of time asleep is recorded every 1/2 hour during the night.

b. Physicians treat patients in twice weekly individual psychotherapy (and five times weekly in group therapy). Observations are recorded and patients are rated on global items after each therapy hour, and on the Brief Psychiatric Rating Scale weekly. Weekly two hour clinical case presentation conferences are conducted in collaboration with H.A. Meyersberg.

c. Social workers, in weekly therapeutic contact with families and spouses, obtain detailed family histories, genetic, and social data.

d. Patients complete self-rating scales on a 2-hourly basis during selective study periods.

e. Videotape records of selected clinical interviews are obtained for systematic evaluation of clinical material and pharmacological response.

3. Telemetric Activity Study. Quantitation of the level of physical activity is achieved through use of a recently developed telemetric system, including wrist-band radiotransmitters and a 4-channel receiver which supplies continuous read-out of patients' activity counts 24 hours/day (developed in collaboration with Plant Engineering Branch).

4. Electrophysiological. In selected patients, all night EEG-monitored sleep records are obtained in collaboration with J.C. Gillin and R. Wyatt, and studies of cortical evoked potentials are conducted in collaboration with M. Buchsbaum.

5. Biochemical Data.

a. Aliquots of continuous 24-hour urine specimens are collected for measurement of biogenic amines, metabolites, and electrolytes.

b. Serum samples are collected in selected patients for monitoring of drug levels, amines, enzymes in neurotransmitter pathways, and electrolytes.

c. Assessment of metabolites and electrolytes in cerebrospinal fluid is carried out systematically during baseline, drug-free evaluation, and at periodic intervals to assess effects of various treatments. 5-Hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG), the respective metabolites of serotonin, dopamine, and norepinephrine are measured by flurometric, gas chromatographic, and mass fragmentographic techni es in collaboration with the Laboratory of Clinical Science.

The probenecid technique, exploiting the fact that probenecid blocks the exit of acid amine metabolites (5HIAA and HVA) from CSF, is utilized to study metabolite accumulation in CSF and thus provide a more dynamic measure of central amine function or "turnover."

6. Pharmacological Studies. Antidepressants and antipsychotic agents are administered on a "double-blind" basis utilizing identical placebo substitution. Mechanisms of action of drugs of proven therapeutic efficacy as well as new, unproven agents with relatively specific effects are explored in this paradigm.

7. Other Somatic Therapies. Evaluation of the effects and mechanism of action of electroconvulsive shock therapy, sleep deprivation, and acupuncture (in collaboration with L.K. Ng) are being explored.

8. Animal Models.

a. A primate facility is being developed in collaboration with M. Ebert for the study of 1) new methods of assessing neurotransmitter function in man; 2) clarifying the interpretations of data derived from techniques currently employed in patients; 3) developing model "psychoses," such as cocaine-induced stereotyped behavior, so that underlying biochemical mechanisms can be evaluated acutely and chronically. (Patients' administering cocaine chronically develop a paranoid, schizophreniform psychosis often with repetitive, stereotyped behavior to which the disturbed monkey behavior may be analogous.)

b. Studies of rodent stimulant-induced activity, stereotyped behavior, and seizures and their underlying biochemical determinants are also conducted employing precise infrared photosensing devices and behavioral measurements.

Major Findings

(1) Intensive longitudinal study of a patient with regular cycles between mania and depression has for the first time emphasized slow biochemical and behavioral changes preceeding, and providing the necessary substrate for, rapid, dramatic behavioral switches occurring in relation to dynamically significant evelnts. Telemetric-measured activity accurately differentiates manic and retarded depressed episodes, and slowly, but not completely,

normalizes after marked increases and decreases at the onset of manias and depressions, respectively. These slow changes are replicable and predict subsequent behavioral change.

The slope of evoked potential also appears to predict clinical change; in this case, decreases in slope preceed switches into either phase. Of the sleep variables studied, rapid eye movement density (a measure of the intensity of eye movements during REM sleep) is almost 100% predictive of clinical state--greater REM densities are associated with depression. Marked fluctuations in REM latency are correlated with and preceed changes in clinical state. Contrary to previous reports in literature, changes in urinary MHPG appear to follow, not preceed, behavioral shifts. Peak values occur one week after manic or depressive shifts. MHPG does not appear to be an artifact of activity as telemetric measures do not correlate significantly with MHPG.

(2) Consistent significant decreases in CSF and plasma calcium have been demonstrated for the first time following electroconvulsive shock therapy. Decreases in calcium are also related to other spontaneous and pharmacologically induced antidepressant responses. Change in calcium levels during lithium therapy are correlated with and predict clinical response.

(3) Contrary to predictions, clonidine, a drug with potent norepinephrine receptor stimulating properties, does not appear to be a useful antidepressant or antipsychotic agent but may exacerbate specific schizophrenic symptoms.

(4) In endogenously depressed patients, one night's sleep deprivation has transient, but clear antidepressant effects. Positive mood response to sleep deprivation, but not CSF amine metabolite change, is highly correlated with subsequent therapeutic response to basic treatments such as tricyclics, antidepressants, lithium, and ECT. The diagnostic and predictive aspects of the sleep deprivation response may prove to have clinical utility.

(5) Melatonin, a pineal hormone reported to have antidepressant properties has, to the contrary, been demonstrated to exacerbate depressive symptomology--an effect which appears to correlate with increases in serotonin turnover.

(6) Selective clinical investigations of the effects of cocaine are continuing, especially the measurement of cocaine levels in plasma (in collaboration with R. Hawks). Review of the work and literature to date indicates that cocaine, while not a clinically effective antidepressant, is of considerable theoretical importance in that it may be associated with manic-like euphoria, depressive-like dysphoria, and a non-toxic, paranoid psychosis difficult to distinguish from acute schizophrenia. Drug administration variables, interacting with predisposing genetic, personality, biological, and acute stress factors appear to interact to predict the type of clinical response observed. Study of the biochemical correlates of this cocaine model psychosis may yield clues to determinants of endogenous psychoses.

Marked behavioral changes in non-human primates have been correlated with changes in dopamine metabolism in preliminary experiments with chronic cocaine administration and CSF sampling. An inhibitor of catecholeamine synthesis (AMPT) has been shown to block cocaine-induced hyperactivity and stereotyped behavior in rats, as does treatment with physostigmine, an acetylcholine esterase inhibitor, reported to be effective in suppressing mania in man.

(7) Ongoing studies of central amine metabolism as reflected in CSF metabolite levels (in collaboration with F.K. Goodwin and E. Gordon) have confirmed and extended prior work and reveal several new findings.

a. In acute schizophrenics (studied in collaboration with E. Fink and W. Carpenter), baseline 5HIAA, HVA, and MHPG do not differ significantly from that in patients with affective illness or controls. Probenecid-induced accumulations of 5HIAA and HVA likewise are not different from patients with manic or depressive illness. Preliminary data, showing decreases in catecholamine metabolites on recovery are suggestive, however.

b. Clinically effective neuroleptics, such as thorazine and thioridazine, increase dopamine turnover acutely, probably on feedback basis secondary to dopamine receptor blockade. However, chronic phenothiazine administration (more than three weeks) is not associated with such an increase. These time-dependent effects described for the first time may correlate with and explain the time course of onset of clinical response--an immediate effect related to receptor blockade and a two to three week lag before full effectiveness is achieved related to the return of dopamine turnover toward normal.

c. Clinically effective antidepressant treatments such as lithium, amitriptyline, imipramine, and electroconvulsive shock therapy, tend to decrease serotonin turnover.

d. Depressed patients continue to demonstrate lower levels of MHPG in CSF than all other groups studied, including normal parents of neurological patients, neurological patients (studied in collaboration with T.N. Chase), schizophrenic, and manic patients. Moreover, levels of MHPG do not increase after clinical improvement with pharmacological or ECT treatment or in a small group of patients studied off medication. This suggests that decreased MHPG may be state-independent and represent an underlying biological predisposition to depression rather than a correlate of depression itself.

Proposed Course of Project

During the next year emphasis will be placed on continued refinement of techniques for measurement of alterations of mood, cognition, motor activity, and neurotransmitter metabolism in patients with acute psychoses.

Continued focus will be placed on the prediction of therapeutic response on the basis of clinical, biological, and pharmacological test-dose profiles. Acute response to stimulants, such as methylphenidate and amphetamine, will be explored in this regard. Particular attention will be given to elucidation of

role of neurotransmitter receptor function and how alterations in receptor sensitivity with specific pharmacological treatments may affect behavior. Further delineation of the interaction of genetic, experiential, and biological factors in the onset and treatment of the acute psychoses will be sought. The idea that behavioral change may be correlated with multiple biochemical alterations and feedback adjustments which have specific temporal courses and developmental sequences rather than a single biochemical abnormality determining behavior will be highlighted. Thus, the interaction of biogenic amine function with that of acetylcholine and other transmitter systems will be explored. Attention will be given to study and treatment of drug-related psychoses in patients and the development and elucidation of pharmacological and animal "model psychoses."

Honors and Awards

Dr. Post discussed "Cerebrospinal fluid MHPG in affective disorders: State dependent vs. state independent variables" at the American College of Neuropsychopharmacology meeting in Palm Springs, California. He was invited to present a paper on "Psychopharmacology: Clinical applications" to the American Society of Experimental Therapeutics in Philadelphia, Pennsylvania. At the Annual Meeting of the American Psychiatric Association in Detroit, Michigan, he will present a paper on "Multiple psychiatric syndromes with a single drug."

Dr. Post is also the co-author on four other papers to be presented at this meeting, including studies of cerebrospinal fluid amine metabolites in acute schizophrenia and its treatment as well as in manic-depressive illness and its treatment.

Dr. Carman will present a paper entitled, "Calcium, ECT, Lithium and Mood" at the Annual Meetings, American Psychiatric Association, May, 1974, in Detroit, Michigan.

Dr. Stoddard will also present a paper entitled, "Phasic changes in manic-depressive illness" at this meeting.

Publications:

Post, R.M., and Goodwin, F.K.: Simulated behavior states: An approach to specificity in psychobiological research. Biol. Psychiat., 7:237-254, 1973. (A.E. Bennett Award)

Post, R.M., Gillin, J.C., Goodwin, F.K., Wyatt, R.J., and Snyder, F.: The effect of orally administered cocaine on sleep of depressed patients. Psychopharmacologia, in press 1974.

Post, R.M., and Goodwin, F.K.: Effect of amitryptyline and imipramine on 5HIAA and HVA in cerebrospinal fluid of depressed patients. Arch. Gen. Psychiat., 30:234-239, 1974.

Post, R.M., and Goodwin, F.K.: Cerebrospinal fluid amine metabolites in affective illness. In Karger, S. (ed.): Proc. of the 2nd Congress of the International College of Psychosomatic Medicine. AG, Basel, Switzerland, 1974, in press.

Post, R.M., Kotin, J., and Goodwin, F.K.: Cocaine: Clinical effects in depressed patients. Amer. J. Psychiat., 131: 511-517, 1974.

Post, R.M., Allen, F., and Ommaya, A.: Cerebrospinal fluid flow and ^{131}Iodide transport in the spinal subarachnoid space. Life Sciences, 14, 1974, in press.

Gillin, J.C., Post, R.M., Wyatt, R., Goodwin, F.K., Snyder, F., and Bunney, W.E., Jr.: REM inhibitory effects of L-DOPA infusion during human sleep. Electroenceph. Clin. Neurophysiology, 35: 181-186, 1973.

Post, R.M., Kotin, J., Goodwin, F.K., and Gordon, E.K.: Psychomotor activity and cerebrospinal fluid amine metabolites in affective illness. Amer. J. Psychiat., 130: 67-72, 1973.

Kotin, J., Post, R.M., and Goodwin, F.K.: Drug treatment of depressed patients referred for hospitalization. Amer. J. Psychiat., 130: 1139-1141, 1973.

Goodwin, F.K. and Post, R.M.: The effect of antidepressant treatment on central serotonin turnover in depressed patients. In Costa, E. and Gessa, G.L. (Eds.): 5-Hydroxytryptamine in Brain. Raven Press, 1973.

Serial No. M-CR-10(c)
1. Child Research Branch
2. Child Behavior
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Relations of Preschool Behavior to Present and Earlier Parent and Child Characteristics

Previous Serial Number: SAME

Principal Investigator: Charles F. Halverson, Jr.

Other Investigators: Mary F. Waldrop

Cooperating Units: Department of Educational Psychology, State University of New York at Albany

Man Years:

Total: 7.4
Professional: 2.0
Other: 5.4

Project Description:

Objectives:

To organize and describe patterns of behavior and individual differences in young children through experimental and naturalistic studies in the early preschool years; to illuminate some of the determinants of these behavioral patterns by relating findings to data provided by other research teams in the Branch longitudinal program.

Methods Employed:

Data collection for this project on preschool behavior was begun in October of 1969 and was completed in January of 1974. During over four years of data collection a total of 199 subjects were seen in some phase of the nursery school operation -- 96 males and 103 females. The core sample consisted of 132 preschool children, 65 males and 67 females, each of whom was seen in a half-day study of play and attachment behavior and in a four-week nursery school session. The additional 67 subjects were seen only during the half-day visit in which measures similar to the four-week nursery school were employed. These subjects were seen because they were unable, for various reasons, to attend the full four-week session of the nursery school. Of these 67, 45 (21 males and 24 females) were second-born offspring of couples who

were in the original core sample of 132 subjects. For all subjects, age ranged from 2 years, 4 months to 3 years, 6 months, with a mean of 2 years, 11 months.

The basic procedure of the preschool studies consists of having children in groups of five attend nursery school for four weeks, where behavioral measures based on direct observation and ratings of free play are obtained. Broadly, our interest has been focussed on attentional deployment in the various settings of free play, on social behavior towards peers and caretakers, and in the nature of assertive and coping behavior elicited in naturalistic play settings and in the various experimental situations. Data for the project can be broken down into six categories:

1. Ratings done both from a half-day visit prior to nursery school and during the child's attendance at the four-week nursery school.

2. Observational data coded by observers from behind one-way vision glass daily during the child's attendance.

3. Experimental settings that elicit coping and assertive behaviors.

4. Standardized testing of cognitive and intellectual performance.

5. Data on current family life recorded in the home by the mother in detailed diaries over a three-week period.

6. Interview data derived from three separate maternal interviews.

All core rating and observational data have been coded and entered on computer tape. Data from the experimental settings is still being coded from video tapes and analyzed prior to final data reduction.

Major Findings:

An analysis of the reliabilities of teacher ratings and observational codes has been recently completed. Reliabilities of 48 teachers' ratings were .66 to .96, with a mean of .85. Ninety eight observational codes were found to have inter-observer reliabilities ranging from .08 to .99, with a mean of .74. Fifteen will be dropped from subsequent analyses. Further measures may be dropped when stability analyses are completed. For longitudinal analyses we will retain only variables with both satisfactory observer agreement indices and high stability over time.

Initial reduction of reliable teacher ratings by principal components factor analysis has revealed 11 orthogonal factors for both males and females, summarizing such domains as peer behavior, excitability, play involvement, assertive behavior, speech development, fearfulness, and hyperactivity. These factors account for a major amount of the common variance in the teacher ratings. Reduction of observational data has only just begun. Preliminary checks indicate that many variables are significantly different for the sexes

and show significant correlations with age at time of attendance. Additional corrections for skewness and other forms of non-normal distribution will have to be performed before observational data are suitable for further analysis. Codes for the diary data kept by the mother on three separate weeks have been analyzed for stability over the weeks the mother coded the data. The stability for the 16 variables coded from these diaries ranged from .40 to.92 with a mean of .80, indicating considerable stability of the home setting variables.

Rating data collected on the half-day visit has been compared with rating data summarizing the four-week attendance of the child in order to assess the extent to which behavior from the brief visit is predictive of four-week nursery school behavior. Our initial variable-by-variable checks have determined that there is considerable stability between the two periods. Sex differences are apparent in that the half-day measures predict peer behavior for females and assertive, high intensity behavior for males. High predictability is obtained for both sexes on speech development and verbal originality. Thus, to a certain extent we may be able to recover from the data of the half-day study, data lost due to longitudinal subjects who could not attend the four-week school.

Several reports have been presented at scientific meetings, and one has been accepted for publication. Gale Inoff of our section has reported on the reaction to unfamiliar adults in the half-day study of our main cohort of children. She found that (1) behavioral measures of reaction to unfamiliar adults were intercorrelated in a pattern we termed "disruption of activity," (2) girls showed more disruption of activity than boys, and also discriminated between male and female strangers more than boys, and (3) there are indications that the disruption of activity is short-lived and that the concept of fear needs broadening and clarification if it is to be applied to children as a whole. It was hypothesized that girls are more friendly and socially oriented when they are comfortable, but their social orientation makes them more attuned to the unfamiliar person and his behavior. They are more apt to stop, look, and find out what the stranger is doing. Their responses may reflect a higher degree of alertness to changes in the environment. Such alertness could be interpreted as reactive fear but it also could be interpreted as indicating a higher level of developmental maturity. Further analyses will continue on the domain of attachment behaviors shown on the half-day visit and their behavioral correlates in the four-week nursery school. Antecedents in the earlier longitudinal material will be assessed as well.

In a paper to appear in Developmental Psychology we have reported on the relations between coping behavior in the nursery school and coping behavior as assessed by a variety of measures five years later in a follow-up study. Experimental barrier settings provide opportunities for children to show how they deal with frustrating blocks to ongoing behavior in their own characteristic way. Their attempts can be organized and quantified with some precision. Measurement of barrier behavior makes it possible to tap a complex and extensive network of interrelated and important personality dimensions.

Coping behaviors showed stability over the five year period. Preschoolers

who coped better with an experimental barrier situation at 2½ were, at 7½, the children assessed as brighter and more imaginative, as coping better with novelty, as being more at ease socially, and as showing more initiative with their peers. Generally, these data seem to indicate that children who demonstrate an ability early in life to be agents capable of producing change in their environment appear in middle childhood to be active, vigorous, and competent. Similar dimensions and barrier situations have been included in the main cohort to assess the domain of active, vigorous, and competent behavior.

Significance to Bio-medical Research and the Program of the Institute:

The material collected in this project is yielding basic information concerning the development of social, emotional, and cognitive behavior patterns in the young child. Results should help in understanding the child's attachment towards his parents, and also generally provide information concerning personality dynamics in early childhood. The unique data provided by the longitudinal project should assist in planning services in the mental health area. The preschool setting could become one of our most important mental health screening devices.

Proposed Course of the Project:

Since the project has just recently finished the data collection, only preliminary analyses have been performed. The methodological focus of the analyses will be to seek measures which are stable over the four-week period as well as across the various settings of the nursery school. These measures then will be summarized in multi-method multi-setting composites of both observed and rated behavioral characteristics.

Additional analyses of our data base are planned in the areas of attachment behavior, social behavior, the differences between first-born and second-born children in our nursery school setting, the relations between the incidence of minor physical anomalies and high intensity behavior, and individual patterns of teacher-child interaction. Conceptual and methodological analyses of preschool behavior will provide hypotheses about the antecedents in these developmentally important areas.

Honors and Awards: None

Publications:

Halverson, C. F., Jr. and Waldrop, M. F.: Relations between preschool barrier behaviors and early school-age measures of coping, imagination, and verbal development. Develpm. Psychol., 1974, in press.

Serial No. M-CR-11 (c)
1. Child Research Branch
2. Infant Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Relations between Human Neonatal Behavior and Later Development

Previous Serial Number: SAME

Principal Investigator: Raymond K. Yang

Other Investigators: Richard Q. Bell

Cooperating Units: The nursery facilities of 13 Washington Metropolitan Area hospitals

Work Years:

Total:	3.00
Professional:	1.25
Other:	1.75

Project Description:

Objectives:

This psychophysiological and behavioral study of human neonates is designed to: 1) identify stable measures of individual differences; 2) locate continuities and discontinuities between the neonatal period and later periods of development; and, 3) describe the fashion in which neonatal characteristics interact with parental variables.

Methods Employed:

The data corpus consists of physiological and behavioral recordings obtained from 138 neonates born between 1967 and 1970 and participating in The Bethesda Longitudinal Study of the Child Research Branch. The physiological data consists of continuous polygraphic recordings obtained during two inter-feeding periods (totalling five hours). Heart rate, respiratory rate, skin temperature, skin resistance, and eye movements were measured. The behavioral data consists of ratings of spontaneous motor activity during sleep, and sensori-motor measures obtained with the infant awake. The latter measures include responses to tactile, aversive, sucking, and quieting stimuli. Complete hospital records are also available.

All data were collected in the hospitals where the infants were born. Average age of the infants at testing was 59 hours.

Major Findings:

As indicated in earlier annual reports, fiscal year 1971 and 1972 were spent in primary data reduction. This involved initial approximations of the stability of specific measures and the conversion of data to forms amenable to computer processing. Because of incompatible physiological recording equipment, rapid data processing techniques, generally available for these types of data, could not be used at the level of primary data reduction. Fiscal year 1973 was spent in checking on problems of observer agreement, trend effects in observers over the three year period, and other tasks basic and preliminary to assessment of the meaningfulness of the data.

Data analyses were significantly impeded from February 1973 to November 1974, during which time the Section was unable to hire a research assistant.

The assessment of behavioral stability in the neonatal period is a necessary prerequisite for examining longitudinal relationships between neonatal, infant, and early childhood behaviors. Establishing such stability helps prevent a harvest of random longitudinal relationships, and provides a preliminary basis for eliminating, sorting, and combining measures within the neonatal period. Assessment of the stability of all single measures in the data corpus has essentially been completed. Many of last year's analyses have been repeated because it was found that corrections for skewness improved the stability of some measures. Maintaining acceptable levels of stability as single measures were: sucking responses to a non-nutritive nipple; crying behavior resulting from withdrawal of the sucking stimulus, and; crying responses to aversive stimulation. Performance on a measure of gross muscle strength (the prone head response) exhibited stability only for males. Not demonstrating stability was quieting behavior (i.e. cessation of crying) following soothing; the determination of tactile thresholds during active and quiet stages of sleep, and; latency of response to withdrawal of the sucking stimulus. A suitable test of stability for a sucking discrimination procedure has not yet been determined.

The inability of specific single measures to exhibit acceptable levels of stability does not preclude their use. These measures can be used in composites rather than singly in cross-stage analyses,provided that ccmposite stability can be demonstrated. They may also be useful in cross sectional descriptions of the neonatal period. As an example of a cross sectional description of the neonatal period, Yang and Douthitt have published an analysis of behavioral and heart rate responses to threshold levels of tactile stimulation, a measure which in and of itself, did not demonstrate stability.

The behavior of the neonate can be viewed from two standpoints. The newborn may be seen as an "end-point," that is, as an outcome of prenatal and maternal factors. For example, the maternal interviews conducted by the

Section on Parent-Infant Behavior during the first and third trimesters of pregnancy could provide gross information regarding the mother's adaptation and physiologic interaction with the developing fetus. Relationships here might involve subtle effects mediated by maternal and fetal hormonal exchange, or something as direct as the mother's response to "quickening." Another, but a more likely possibility, involves the relationships between maternal attitudes assessed by pregnancy interviews and the conduct of labor and delivery. Complications of labor and delivery (subclinical variation included) have demonstrable effects on neonatal behavior. Events occurring during labor and delivery could provide a bridge by which maternal attitudes expressed during pregnancy could relate to neonatal behaviors. And, within a shorter time span, the conduct of labor and delivery could be viewed as an antecedent event with neonatal behavior a consequence.

Some empirical justification for exploration along these lines was provided by a preliminary analysis of analgesic medication during labor and maternal attitudes expressed during the third trimester of pregnancy. Mothers high in generalized anxiety (measured by questionnaire) were found to receive significantly more analgesic administrations during labor than mothers low in generalized anxiety. In a related preliminary analysis, we were however, unable to demonstrate any substantial relationship between amount of medication during labor and depressed neonatal behavior. However, this does not mean that the medication during labor had no effect on the infant. Other studies have reported finding effects in later infancy. We plan to check these in our data.

The second standpoint is the more traditional of the two and views the neonate as a "beginning-point." Here, the major thrust is toward establishing sets of stable behaviors whose continuity can be traced longitudinally to later ages. It is this approach that was taken in Bell, Weller, and Waldrop's monograph report on their newborn-preschool follow-up. They reported paradoxical shifts in relations between measures obtained at the neonatal period and during the preschool period. These relations were collectively characterized as a "longitudinal inversion of intensity." The Section on Infant Development and the Section on Child Behavior are currently engaged in an attempt to conduct an essential replication of the "inversion of intensity" hypothesis. The replication is proceeding in two steps. The first step involved an attempt to create variables in the data sets available to the Section on Infant Development and the Section on Child Behavior that are identical to the Bell et al. measures. These variables, being selected for their relevance to the inversion of intensity hypothesis, would be related between periods in a fashion identical to the Bell et al. study. This step has been essentially completed, and the inversion hypothesis does not appear to be supported. However, the actual test of the hypothesis by correlational analyses between the newborn and preschool period was somewhat compromised by our inability to demonstrate stability for one of the five newborn measures. Furthermore, there are major differences between various components of the current longitudinal sample and the Bell et al. sample. For example, the current longitudinal sample, unlike Bell et al., is composed entirely of first born children. There is also the possibility that the two samples differ in

terms of socio-economic status. These and other factors will require a thorough investigation before formal analyses can be considered as having been completed. We also intend to conduct a more conceptually based test of the inversion hypothesis. Rather than focusing on the replication of relationships between specific measures identical to those used by the Bell et al. study, we are organizing selected measures currently available in the newborn and preschool data sets that might represent an "intensity-activity" dimension.

The Section on Infant Development was also given the opportunity to prepare a book chapter reviewing and evaluating currently available tests of infant development. The chapter, covering what the authors felt to be the most widely used and most promising tests, had the direct benefit to our section program of bringing us up to date on the most recent longitudinal studies that have been carried out in order to validate developmental tests that have been extended down to the neonatal period.

Significance to Biomedical Research and the Program of the Institute:

Should relationships be found between maternal attitudes expressed during pregnancy, the conduct of labor and delivery, and neonatal behavior, bases would be laid for more effective perinatal management techniques. Concomitantly, should longitudinal relationships between neonatal and early child behavior be established, bases would be laid against which postnatal pediatric procedures might be evaluated as to their possible long term effects on the infant.

Proposed Course of the Project:

With the completion of tests of stability for single newborn measures, the Section will be turning toward the characterization of the newborn along behavioral dimensions. This will include factor analytic approaches as well as a priori combinations of measures based on relevant conceptual models. With regard to the latter, our first attempt will be based on a model of "homeostatic ranging" as affected by levels of environmental exchange. Our data set enables us to define minimal, moderate, and intense levels of environmental exchange, and it is in terms of organismic variability during these levels that we initially hope to characterize neonates. These dimensions, along with other variables will serve as the pool from which longitudinal relationships to other variables (in other Sections) will be examined.

Honors and Awards: NONE

Publications:

Yang, R. K. and Douthitt, T. C. Newborn Responses to Threshold Tactile Stimulation. Child Development, 1974, 45, 237-242.

Yang, R. K. and Bell, R. Q. The assessment of infants. In McReynolds, P.W. (ed.): Advances in Psychological Assessment. San Francisco, Jossey-Bass, Inc., 1974, Vol. 3, in press.

Serial No. M-CR-12 (c)
1. Child Research Branch
2. Section on Family Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Developmental Patterns in the Young Family

Previous Serial Number: SAME

Principal Investigator: Robert G. Ryder

Other Investigators: John S. Kafka, David H. Olson and Walter Sceery

Cooperating Units: None

Man Years:

Total:	4¼
Professional:	2
Other:	2¼

Project Description:

Objectives:

To investigate variations in relationships among young married couples; to describe and explicate the developmental history of differing patterns of relationships; to integrate subcultural, social, and personality phenomena into a comprehensive view of human relationships; to investigate correspondence among marriage patterns, attributes of offspring, and patterns of parent child interaction.

Methods Employed:

Most of the present research data were previously collected from a sample of approximately 2000 young married couples from the Washington, D. C. metropolitan area. Final data was collected this year from the last cases in which couples' children were studied in the preschool phase of the Branch longitudinal study. Data collection procedures for all of these cases included observed interaction, questionnaires, and unstructured interviews. Computerized records have been made of most of the quantitative data from this project. Most non-quantitative data has been recorded on microfilm. Data not yet in either of these forms consists of information most recently collected, in the preschool phase, and recently coded data collected somewhat earlier.

Major Findings:

Since the Section on Family Development is being inactivated early in FY 1975, primary efforts have been devoted to generating archival records of data to be available for future work. This will be largely accomplished by the end of this fiscal year. Also, work is in progress to ascertain the present location and status of the couples studied.

A combined analysis, using data from interviews, questionnaires, and observed interaction is in progress, and should yield the beginnings of a dependable dimensional space for describing married couples, which can then be used in longitudinal analyses to relate aspects of marriage to subsequent aspects of child development. Replicated analyses presently completed suggest that stable dimensions will be forthcoming, including dependable interrelationships between observed behavior and self reports, and between each of these and clinical judgments based upon unstructured interviews.

Significance to Biomedical Research and the Program of the Institute:

A growing concern among professionals and in our society at large about the role of the family in maintaining mental health or ill health, indicates that there will be a growing investment in family related research in the years to come. As one of the best known projects in this area, with the most comprehensive body of data, this project and its data are and will continue to be a focus of much professional interest. The existence of archival records is an urgent priority, so the unique and irreplaceable body of data collected by this project can continue to provide returns in the form of information concerning marriage, the family, and antecedents of child development.

Proposed Course of Project:

Dimensional analyses produced this year will be incorporated into studies of infancy and parent-child outcomes by other sections of this branch. The studies of marriage as such, and marriage follow-up data, will continue at university research centers that are collaborating in research on these unique data on the early phase of the human life cycle.

Honors and Awards:

None

Publications:

None

Serial No. M-CR-22 (c)
1. Child Research Branch
2. Infant Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Offspring Effects on Parents

Previous Serial Number: SAME

Principal Investigator: Richard Q. Bell

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.75
Professional:	.50
Other:	.25

Project Description:

Objectives:

To review the literature for evidence of offspring effects on parents; to provide a conceptual scheme to organize findings; to initiate studies in areas crucial to theories of early development.

Methods Employed:

Literature search, concept development, and development of research methods relevant to offspring effects on parents.

Major Findings:

This last year saw the completion of a series of papers that have reappraised the literature on human socialization, as well as studies on early development in other mammals, to determine the extent to which the effects of the young on adults have been underestimated; to see what findings could be reinterpreted as due to the effects of the young; to provide empirical information on the extent to which the young initiate interactions with adults, to identify research approaches that can be used to isolate the effects of each contributor to the developmental process; and to provide an initial theory of how the young contribute to the nature of the caregiving they receive, as well as affect the adult lives of the caretakers, and the

social systems of the broader society in which the young are developing. The last report prepared this year attempted to provide some perspective on how twentieth century science came to neglect the role of the young, by tracing historical data from ancient Greece, through the medieval period in Western Europe, to current developments in the United States. The review collated data indicating the extent to which various societies recognized and regarded as important: (a) the distinctive characteristics of infants and children, (b) individual differences among children of the same age, (c) the power or importance of the young, (d) the effects of the young on their parents or caretakers during specific interactions, and (e) the special contributions of the young to the larger social group, the neighborhood, the community, and society. These aspects were treated in terms of two mediating factors: the segregation or integration of the young with other segments of society, and the diffusion of concepts concerning child influences beyond elite groups of scholars to the general public. It was found that change in these five effects has by no means been progressive through this historical period. In ancient Greece, the importance of children, the distinctive characteristics of immaturity, and individual differences, were all clearly recognized, by the elite at least, while in the medieval period children were treated much as adults, though their special characteristics may have had considerable impact simply as a function of the complete and open exposure of the adult world to the young. During the Reformation, greater importance was attributed to the young, but children were increasingly insulated from the rest of society, and their distinctive characteristics suppressed. The writings of the early diarists, and of Locke and Rousseau, as well as the revolutionary movements against monarchies and hereditary determination of position, again altered the status accorded childhood.

Of particular importance to scientific recognition of the importance of children was (a) the impact of evolutionary theory, and its emphasis on the child as a repository of instincts, and (b) psychoanalytic theory in its representation of the child as an antagonist of civilization. While these various developments resulted primarily in three schools of thought that had impact on the public (the "break the will" school, the "hardening" school, and the "gentle treatment" school), the most significant effect of scientific work of the twentieth century seemed to have issued from the negative reaction of sociology and psychology, after an initial period of incautious enthusiasm, to the biological approach represented in evolutionary theory. The child's contribution to development became equated with biological contributors, and rejected by modern behaviorism, along with other extensions of evolutionary theory. The Hullian psychoanalytic studies of socialization, and Piaget's biological-epistemological approach brought the child to the forefront of research attention again, but it was primarily the failures of a one-sided research approach to socialization, neglecting the effects of the children, that have brought to the attention of scientists the necessity of treating parent and child as a social system.

Significance to Biomedical Research and the Program of the Institute:

This series of conceptual, methodological and empirical contributions is intended to bring about a revision of socialization theory and research methods that have, in the main, proved relatively unproductive in recent years. The revised approach may make it possible to launch socialization research on a course that corresponds better to the realities of parent-child interaction, and thus increase the likelihood of generating more consistent results than in the past. Socialization research is, in turn, the basis for advice to parents as well as the basis for many social and family-oriented action programs.

Proposed Course of the Project:

This series of studies was launched by findings in early case studies, from the branch longitudinal project, indicating a much more important role of the young in affecting marriages and parents than had previously been recognized. Most of the theoretical work and literature review was carried out during a period when the longitudinal project was in active data gathering, and samples were insufficient to test hypotheses concerning the effects of the young on adults. The conceptual framework and methods that have been developed can now be brought to bear on the data from the longitudinal project. Thus, further work on the effects of the infant and child will appear in empirical reports on the longitudinal project, and this project will be discontinued.

Honors and Awards:

Dr. Bell has been invited to present a report on the above research to an international group of scientists specializing in studies of parent-child interaction, to be convened by the CIBA Foundation in London, 1974.

Publications:

Bell, R. Q. Theoretical and methodological limitations imposed by an exclusive search for consistency of personality. In Proceedings of the Twentieth International Congress of Psychology, August 13-19, 1972, Tokyo, Japan: University of Tokyo Press.

Bell, R. Q. Contributions of human infants to caregiving and social interaction. In Lewis, M. and Rosenbloom, L. A. (Eds.): The Origins of Behavior: the effect of the child on the caregiver. New York, Wiley, 1973.

Serial No. M-CR-23 (c)

1. Child Research Branch
2. Section on Parent-Infant Behavior
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Determinants and Dimensions of Mother-Infant Interaction

Previous Serial Number: Same

Principal Investigator: Howard A. Moss

Other Investigators: None

Cooperating Units: None

Person Years:

Total:	2.75
Professional:	1.00
Other:	1.75

Project Description:

Objectives:

The major objectives of this research are to identify primary dimensions of mother-infant interaction, to investigate the determinants of these interaction patterns, and to identify the degree to which these patterns contribute to subsequent infant and child behaviors.

Methods Employed:

The data collection for this project has been completed and we currently are engaged in data analysis and reporting of findings. The sample consists of 121 mother-infant pairs (64 male and 57 female infants) from the second cohort of the Branch's longitudinal program.

All the procedures administered in the main longitudinal study of mother-infant interaction have been described in detail in previous annual reports. Briefly, these consisted of pregnancy interviews and questionnaires, 3-month home observations, laboratory procedures, maternal interviews and questionnaires, and an 11-month home visit covering structured and free play behavior. In addition, we have obtained home observation data on a subsample of 32 second-born mother-infant pairs for whom we have equivalent data for the same mother and their first-born sib. This subsample has made it possible for us to determine differences in maternal behavior toward first- and second-borns, using the mother as her own control.

The home observations were coded for 41 variables involving either maternal or infant behaviors. In turn, these 41 variables have been combined in various groupings to produce an additional list of 56 combination variables, either through summarizing conceptually related individual measures, or through deriving new measures based on ratios. Ratios have been developed in order to control for the base rate of an infant behavior which might be a factor influencing the maternal output of a particular behavior.

The total number of variables from all of our procedures is unwieldy, both from a statistical and from a theoretical viewpoint, and thus we are interested in reducing the initial sets of variables. This is being accomplished by discarding variables with inadequate measurement properties (i.e. low reliabilities), selecting the more statistically robust variables from among redundant measures, and utilizing summarizing procedures such as factor analysis.

The pregnancy interview variables were subjected to a principal components factor analysis, in order to examine the number of underlying dimensions. Four rotated (varimax) pregnancy interview factors emerged. Summary descriptions of these four factors are as follows:

Factor 1. Positive orientation toward infants and toward the maternal role.

Factor 2. Fearfulness and sense of inadequacy.

Factor 3. Predisposition toward providing and seeking social and physical stimulation.

Factor 4. Establishing close and affectionate ties with family members.

Composite scores derived from these factors were correlated with home observation variables from the third month postpartum. Maternal attitude factors were significantly correlated with functionally similar maternal behaviors, but only for the upper SES subjects, as had been found for the pregnancy interview variables treated singly in the analyses reported last year.

Previous experimental studies on operant conditioning have shown that certain infant behaviors (vocalization and smiling) can be experimentally increased through contingent reinforcement. The important theoretical question that remained to be answered is whether contingent reinforcements that occur during the natural course of events are actually of any significance in shaping infant behaviors in vivo. One way of testing this is by determining whether the frequency of contingent maternal behaviors that are observed under naturalistic conditions is associated with the infants' operant behavior under baseline and reinforcement conditions in the laboratory. We tested this hypothesis by correlating three-month home observation variables involving vocalization with infant responses in a laboratory social conditioning procedure carried out when the infants were about 3½ months of age. It was found that the frequency of contingent maternal behaviors toward infant

vocalizations in the homes was moderately predictive of differences among infants in their vocalization rates during vocal reinforcement in our laboratory. This relationship was strongest for females, whereas the tendency to exhibit similar vocalization rates in both the home and the laboratory, irrespective of reinforcement contingencies, was more characteristic of the male infants.

Last year we reported some initial results comparing the sample of second-born cases we studied with their first-born sibs on the three-month home observation variables. The mothers in our study showed much less interaction, both social and caretaking, with their second-borns than they did with their first-borns. These analyses have now been carried further. Results based on sex by birth order comparisons revealed that the decrement in maternal treatment toward the second-born was greatest for female second-borns with older sisters and next greatest for female second-borns with older brothers. Male second-borns were less affected as a function of their birth order. In particular, we found no decrease in maternal attention for male second-borns who had first-born sisters. These data also gave us the opportunity to evaluate the generality and consistency of maternal behavior by correlating the mothers' behavior toward their first- and second-born offspring who were separated in age by an average of 25 months. This is one way of estimating the mother's contribution to the interaction, since two infants in the same family often vary greatly. We found that mothers tended to be highly similar toward both infants for the following behaviors: use of physical affection, physical distance maintained from the infant, use of a pacifier, and frequency of imitating infant vocalizations. These correlations ranged from .45 to .74 and all were beyond the .01 level of significance. As far as we know, these are the first data throwing light on maternal consistency in this early age range.

Significance to Bio-Medical Research and the Program of the Institute:

The data collection from this project should provide basic information concerning the extent to which early response tendencies in infants, and various parental characteristics, conjointly-contribute to early mother-infant interaction patterns. From these data we can begin to determine the variance in the mother-infant interaction that is attributable to infant characteristics, as well as the variance associated with particular maternal predispositions. Almost all experts in the field of child development would agree that this kind of information, all too rarely available from past research, is vital to a more solid empirical base for theories of mental health. From information on the conjoint contribution, we should reach an improved understanding of the mother's and infant's attachment to one another; the emergence of social behavior and responsiveness in the child, and some of the developmental antecedents of cognitive functioning. The results should also provide information concerning the basic dynamics of early personality functioning.

Proposed Course of Project:

We are currently in the midst of summarizing our various sets of data through factor analyses. We will derive a final set of factor scores for the pregnancy interview materials, the three-month home observations (separate

factors for maternal and infant behaviors), the laboratory studies of the infant, and the postpartum interviews. It is possible that some of these sets of data will be combined (e.g. infant observation and infant laboratory) in order to derive a more general set of composite scores. However, this decision will have to await the findings from the preliminary analyses. One example of the types of cross-stage analyses we will be conducting is to evaluate the degree and manner in which contingent maternal behavior, assessed during the infancy period, shapes pre-school socialization and verbal development.

Honors and Awards:

None

Publications:

Moss, H. A. Communication in Mother-Infant Interaction. In T. Alloway, L. Krames, and P. Pliner (Eds.), Nonverbal Communication: Comparative Aspects. New York: Plenum Press, In Press.

Moss, H. A. Early Sex Differences and Mother-Infant Interaction. In R. C. Friedman, & R. M. Richart (Eds.), Sex Differences in Behavior. New York: Wiley, In Press.

Moss, H. A., & Jones, S. J. Relations between Maternal Attitudes and Maternal Behavior as a Function of Social Class. In P. H. Leiderman, & S. R. Tulkin (Eds.), Cultural and Social Influences on Behavior in Infancy and Early Childhood. Stanford: Stanford University Press, In Press.

Serial No. M-CP(C)-18-3
1. Laboratory of Clinical Psychobiology
2. Section on Clinical Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical and Pharmacological Studies of Sleep

Previous Serial Number: Same

Principal Investigator: Frederick Snyder, M.D.

Other Investigators: J. Christian Gillin, M.D., David H. Fram, M.D., Richard J. Wyatt, M.D., Robert I. Henkin, M.D., Bernard L. Frankel, M.D., Dennis Murphy, M.D. and Daniel P. Van Kammen, M.D.

Cooperating Units: Section on Psychiatry, Laboratory of Clinical Science, DCBR; Laboratory of Clinical Psychopharmacology, SMHR; Experimental Therapeutics, NHLI

Man Years (1973):

Total:	3/4
Professional:	1/4
Other:	1/2

Project Description:

Objectives:

Experimental findings over the past decade promise understanding of the basic biochemical processes underlying sleep and increasingly clear demonstrations of the severity of sleep disturbances in many psychiatric patients indicate the need for better means of alleviation. To whatever extent biochemical understanding of sleep can be achieved, more effective treatment of its disturbance will surely follow.

Using various biochemical and pharmacologic techniques in normal controls and patients with different disorders, we have previously found that both serotonergic and adrenergic systems as well as carbohydrate active steroids appear to influence the occurrence of REM sleep. The relationship of delta (slow-wave) sleep to these systems or to diurnal changes in various hormone secretory rates is much less clear. While a more concentrated effort to pursue these issues is being carried on by 2 of our

alumni, Drs. Richard J. Wyatt and J. Christian Gillin, in the Laboratory of Clinical Psychopharmacology, SMHR, several specific questions have been the basis of continued collaboration here during the past year.

Patient Material:

3 narcoleptic patients
4 psychiatric patients

Methods and Findings:

A. Relationship of Cholinergic Activity to REM Narcoleptic Attacks

In order to explore the possible relationship between levels of cholinergic activity and REM narcoleptic attacks, Drs. Gillin and Frankel have begun therapeutic trials of the cholinergic blocking agent, Artane (trihexyphenidyl 2 mg, tid). Results from the first 3 narcoleptic patients have been encouraging in that all 3 appeared to show clinical improvement, especially in terms of less frequent attacks of cataplexy. The converse procedure of administering physostigmine (0.5 mg i.v., q 5 minutes to a total dose of 1.5-2.5 mg) in an effort to determine whether increased cholinergic activity induces REM narcoleptic cataplexy did not have any discernible effects in the same 3 patients. In addition, physostigmine had no apparent effects on the mood of these patients, contrary to recent reports involving psychiatric patients.

B. Effects of L-histidine on Sleep

There is considerable indirect evidence suggesting that histamine may function in the brain as a neurohumor in sleep-waking mechanisms. To test the possible role of histamine in the control of waking in man, we administered its amino acid precursor, L-histidine, to human subjects and evaluated their electrographic sleep patterns. L-histidine was given to 3 patients with intractable narcolepsy (20 gm per day for 2 weeks), to 4 normal volunteers (32.4 gm per day for 5 days) and to a patient with progressive systemic sclerosis (48.6 gm per day for 16 days). No effects were observed on nocturnal EEG sleep patterns in any of the subjects or on the symptoms of the narcoleptic patients. Although the degree to which histamine levels in the brain were elevated in this study is not clear, these results do not encourage the hypothesis that histamine is an alerting or waking factor in the brain.

C. Differential Effects of D- and L-Amphetamines on the Sleep of Depressed Psychiatric Patients on and off Lithium Treatment

Since there is evidence suggesting that d-amphetamine preferentially blocks norepinephrine re-uptake as compared with l-amphetamine, and that Lithium carbonate enhances norepinephrine re-uptake, sleep patterns of

4 depressed psychiatric patients were examined on nights when they had been given d- or l-amphetamine on and off Lithium therapy. The data indicate that both d- and l-amphetamine suppressed REM sleep and REM %, but that only d-amphetamine suppressed total sleep time. Lithium carbonate did not alter the above-mentioned effects of d- or l-amphetamine.

Significance to Mental Health Research:

These studies contribute to the present extensive national and international efforts to unravel the complex relationships of central nervous system biochemistry and physiology to sleep and to mental illness. The studies of narcolepsy, in particular, bear directly on the understanding of these relationships.

Proposed Course of Project:

The histidine and amphetamine studies are now completed and awaiting publication. Both the physostigmine and Artane trials will continue either here or at LCP as suitable narcoleptic patients become available. These and similar studies involving extensive collaboration across organizational boundaries here at NIH certainly will be continued, although no longer under the auspices of this Laboratory.

Publications:

Gillin, J., Fram, D.H., Wyatt, R.J., Henkin, R.I. and Snyder, F.: L-histidine: Failure to affect the sleep-waking cycle in man. Psychopharmacologia, in press.

_____, Jacobs, L.S., Snyder, F. and Henkin, R.I.: Effects of decreased adrenal corticosteroids: Changes in sleep in normal subjects and patients with adrenal cortical insufficiency. Electroenceph. Clin. Neurophysiol. 26: 283-289, 1974.

_____, Post, R.M., Wyatt, R.J., Goodwin, F.K., Snyder, F. and Bunney, Jr., W.E.: REM inhibitory effect of L-dopa infusion during human sleep. Electroenceph. Clin. Neurophysiol. 35: 181-186, 1973.

_____, Jacobs, L.S., Snyder, F. and Henkin, R.I.: Effects of ACTH on the sleep of normal subjects and patients with Addison's disease. Neuroendocrinology, in press.

Awards and Honors: None

Serial No. M-CP(C)-18-8
1. Laboratory of Clinical Psychobiology
2. Section on Clinical Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychophysiological Studies of Sleep and Waking in Normal Subjects

Previous Serial Number: Same

Principal Investigator(s): Frederick Snyder, M.D. and Bernard L. Frankel, M.D.

Other Investigators: Ms. Evelyn L. Elwell and Ms. Rona Buchbinder

Cooperating Units: Surgery Branch, National Heart and Lung Institute

Man Years (1973):

Total:	2.0
Professional:	0.5
Other:	1.5

Project Description:

Objectives:

The 2 studies in this project share the purpose of seeking sleep-related correlates of stressful situations or processes. The first investigation, undertaken by Ms. Elwell, studies the changes in sleep patterns and mental functioning of patients after open-heart surgery. The second study, being carried out by Ms. Buchbinder, focuses on the changes in sleep patterns in relation to the phases of the normal menstrual cycle.

Patient Material:

5 open-heart surgery patients
8 normal subjects

Methods and Findings:

A. Polygraphic Studies of Sleep in Cardiotomy Patients

Five male patients, ranging in age from 42-59 years were studied to assess their sleep patterns before and after open-heart surgery with cardiopulmonary by-pass. The patient's sleep was recorded for 3 consecutive nights prior to surgery, continuously for an average of 42-hours while in the recovery room, and for 4 consecutive nights after leaving the recovery room. Not surprising in men of this age range under these circumstances, sleep was found to be relatively brief, shallow and fragmented by awakenings even prior to surgery. None of the patients exhibited stages 3 or 4 sleep before or after the operative procedure, and preoperative levels of REM sleep were low in most cases. During the 2-3 days they spent in the recovery room sleep was extremely superficial, broken and, in all but 1 case, very scant. The average interval of continuous sleep in the recovery room was less than 9 minutes. There was no REM sleep in any patient, and the average total duration of sleep for 4 of the 5 patients was 2 1/2 hours per day. For unexplained reasons, the fifth patient averaged almost 12 hours of sleep per day in the recovery room in 10 minute fragments. The most unexpected finding was evidence of continued disturbance of sleep for at least 4 nights beyond the recovery room phase. In most cases total sleep continued to be reduced and interrupted by frequent awakenings, with particular reduction of REM sleep. Unfortunately, it was not possible to continue the study long enough to determine when normal sleep returned or whether the REM sleep loss was eventually compensated.

B. EEG-Sleep Patterns in Phases of the Normal Menstrual Cycle

Polygraphic recordings of sleep from 6 normal healthy young women who had regular menstrual cycles and were not taking oral contraceptives were obtained over complete cycles of 35-40 consecutive nights. For comparison, 2 normal young males were also studied for comparable intervals. Unfortunately, from examination of the women's temperature charts, it appeared that 4 of the 6 female subjects happened to be anovulatory during the periods they were studied. Thus, the main purpose of the study, to examine sleep changes in relation to the normal menstrual cycle, has been largely defeated.

Significance to Mental Health Research:

The study of the changes in sleep patterns in open-heart surgery patients is teasing apart the sleep-disruptive effects of routine post-operative medical and nursing procedures from those related more to the inherent stress of major surgery. If post-cardiotomy sleep deprivation and the

associated disturbances in mental functioning are indeed demonstrated, then it may be possible to consider modifications in post-operative care so as to minimize sleep loss and its attendant psychophysiological morbidity. The delineation of the variations of the menstrual cycle-related patterns of sleep was expected to help elucidate the inter-relationships between sleep, hormones, and mental functioning. Although that purpose may not be served very well by the available data, a subsidiary purpose of examining the extent of intraindividual variability of normal human sleep patterns will be.

Proposed Course of Project:

Data analysis for both studies will be completed and the results submitted for publication.

Awards and Honors: None

Publications:

Williams, Jr., R.B., Frankel, B.L., Gillin, J.C. and Weiss, J.L.: Cardiovascular response during a word association test and an interview. Psychophysiology 10:571-577, 1973.

Serial No. M-CP(C)-18-9
1. Laboratory of Clinical Psychobiology
2. Section on Clinical Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychophysiological Studies of Biofeedback

Previous Serial Number: None

Principal Investigator: Bernard L. Frankel, M.D.

Other Investigators: Robert D. Coursey, Ph.D., David E.W. Mott, M.A., Thomas E. Trautmann

Cooperating Units: Department of Psychology, University of Maryland

Man Years (1973):

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives:

The purpose of this project is to further understanding of human psychophysiology by investigating the objective conditions and correlates of learning to control voluntarily specific physiologic functions thought to be regulated autonomically or unconsciously. The method of learning such control involves the use of a biofeedback device which measures and records a subject's physiological parameter, such as electromyographic (EMG) activity, and converts this measure to a tone and/or light which is "fed back" to the subject who attempts to modify the characteristics of the feedback stimulus by altering the particular physiological function being measured.

Patient Material:

25 normal volunteers
3 patients with chronic headaches

Methods and Findings:

A study evaluating the relative efficacy of EMG feedback as a technique for learning to relax was completed. The experimental group consisted of 10 subjects who used feedback of electromyographic activity of the frontalis muscle as an indicator of level of relaxation. There were also 2 control groups of 10 subjects each: in one, they were told to relax as much as possible; in the other, they were given cognitive instructions related to achieving a relaxed state. The results revealed that although EMG feedback does serve to reduce muscle tension when compared with the 2 control groups, it does not appear to be a very powerful technique when used by itself in normal subjects.

Another study focuses on the learning of finger temperature control. It seeks to identify the ways in which varying parameters of (1) the recording preparation, (2) the processing of the measured response prior to feedback and (3) the feedback per se of these responses affect the learning of their control. Each of about 25 NIH normal volunteers participates in 12 half-hour sessions over 2 weeks, during which his task is to learn to raise his finger temperature. The nature of the feedback stimulus (visual versus auditory, etc.) and the type of processing (absolute versus rate of change) are specifically being studied at present.

A third study, still in a pilot phase, involves patients with chronic tension or migraine headaches who are taught to relax their frontalis muscle or raise their finger temperature respectively as a way of decreasing the frequency and severity of their headaches. Of the 2 migraine headache patients, 1 had subjectively assessed a moderate degree of improvement that was correlated with her ability to raise her finger temperature. The complete disappearance of migraine headaches in the second patient appeared to be related to a significant change in the external circumstances of her life rather than to her facility in raising her finger temperature. One patient with chronic tension headaches and serious analgesic habituation, experienced dramatic relief and gave up the extensive use of analgesics mainly as a result of her learning to relax her scalp muscles via EMG feedback.

Significance to Mental Health Research:

As a result of these studies, it is hoped that some biofeedback techniques can be refined so as to optimize learning under varying circumstances and clinical conditions. From a clinical viewpoint, the potential usefulness of biofeedback, perhaps in conjunction with other techniques, in the treatment of psychosomatic disorders is very significant, especially in consideration of its safety, inexpensiveness, and ease of administration.

Proposed Course of Project:

The collection and analysis of data from the NIH volunteers involved in the finger temperature feedback study will be completed. The continuation of the study of biofeedback in the treatment of psychosomatic disorders does not appear likely in view of the reorganization of NIMH Intramural Research Program which is to occur in the near future.

Publications:

Coursey, R.D. and Frankel, B.L.: EMG feedback as a relaxation technique. In The Proceedings of the Biofeedback Research Society Annual Meeting, February 1974, Colorado Springs, Colorado, page 43.

Honors and Awards: None

Serial No. M-CP(C)-18-69
1. Laboratory of Clinical Psychobiology
2. Section on Clinical Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Longitudinal Studies of Sleep and Concomitant Biological Changes in Psychiatric Patients

Previous Serial Number: Same

Principal Investigator(s): Frederick Snyder, M.D. and Bernard L. Frankel, M.D.

Other Investigators: Kenneth Gaarder, M.D., Robert Coursey, Ph.D., David E.W. Mott, M.A., Monte S. Buchsbaum, M.D. and Rona Buchbinder, B.A.

Cooperating Units: Laboratory of Psychology, DCBR
Department of Psychology, University of Maryland

Man Years (1973):

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description:

Objectives:

The main emphasis in this project continues to be on seeking objective psychophysiological correlates of impaired sleep which will provide quantifiable assessments of diagnosis, clinical course, and effectiveness of therapeutic efforts in various psychiatric conditions. The primary emphasis of new work during the past year has continued to be chronic primary insomnia. In addition, record scoring and data analysis have continued on records obtained earlier from depressed, manic and schizophrenic patients.

Patient Material:

12 insomniac patients

Methods and Findings:

The therapeutic approaches being studied focus on helping insomniacs learn to voluntarily achieve states of deep muscular relaxation in order to facilitate the onset of sleep. Twelve chronic sleep-onset insomniacs are being randomly assigned to 1 of 2 treatment protocols: (a) electromyographic (EMG) biofeedback from the frontalis muscle; or (b) autogenic therapy (a combination of sorts of meditation and self-hypnosis). After having his sleep recorded for 5 consecutive nights in the sleep laboratory and undergoing psychological testing, each insomniac comes to the NIH Clinical Center twice weekly for 6 weeks to learn 1 of the 2 relaxation techniques described above. Daily home practice and the keeping of a daily sleep log are also required. One month after the final training session, the insomniac sleeps in the laboratory again for 5 consecutive nights. At present, of the 5 out of the 12 insomniacs who have completed the protocol, the sleep patterns of 2 do not appear to have changed; each of the remaining 3 felt there was definite improvement (subjectively assessed as mild to marked) in their sleep difficulties. The analyses of the sleep-EEG and pre- and post-treatment psychological test data are not yet complete.

The work with insomniacs led to an interesting case study of a middle-aged woman with severe insomnia secondary to restless legs syndrome, a disorder of sensory and motor symptoms in the lower limbs after they have been at rest for a period of time. Neurological evaluation clearly revealed evidence of lower motor neuron disease in this patient. Sleep-EEG studies demonstrated the delay in sleep onset and the interruptions of sleep by the restless legs symptoms.

Another study, comparing the recorded and reported sleep of 18 insomniacs with 18 age- and sex-matched normal sleepers, was completed. In addition to the significant differences in the sleep patterns of these 2 groups of subjects (as detailed in last year's annual report), the psychological test and average evoked response data also clearly differentiate the 2 groups. In comparison to the normal sleepers, the insomniacs were more depressed, more worryingly anxious, less proficient in perceptual-motor skills on the WAIS, and showed lower evoked responses to sound (i.e., they were sensory reducers).

From records accumulated over past years it has been possible to examine 6 longitudinal case studies bearing upon the question of sleep changes during transient episodes of mania. While sleep was impaired in every instance, with special depletion of REM and delta sleep patterns, the overall extent of sleep loss appears to relate to degree of behavioral disturbance. Several cases displayed an interesting pattern of sleep disturbance not previously noted in any psychiatric condition involving nights of little or no sleep alternating with nights of longer than normal total sleep. This study is now being prepared for publication.

Significance to Mental Health Research:

After documenting the sleep and psychological characteristics of chronic insomniacs, we are researching non-pharmacologic treatment approaches which are safe, inexpensive, and relatively simple. Both autogenic therapy and electromyographic biofeedback appear to help some insomniacs to sleep better as well as generally be able to relax more easily. As more experience is gained it appears that we will be able to predict which insomniacs might benefit from these techniques. The potential applicability of these techniques for other psychosomatic disorders merits further investigation. The value of objective information about sleep patterns in various psychiatric conditions is self-evident.

Proposed Course of Project:

The evaluation of the therapeutic effectiveness of biofeedback techniques and autogenic therapy in chronic insomnia will be completed during the next year, as will the scoring and analysis of earlier longitudinal studies of depression and schizophrenia.

Honors and Awards: None

Publications:

Frankel, B.L.: Research on cerebral electrotherapy (electrosleep). Some suggestions. Amer. J. Psychiat. 131: 95-98, 1974.

Frankel, B.L., Buchbinder, R. and Snyder, F.: Ineffectiveness of electrosleep in chronic primary insomnia. Arch. Gen. Psychiat. 29: 563-568, 1973.

Snyder, F.: Electroencephalographic studies of sleep in psychiatric disorders. In Chase, M.H. (ed.): The Sleeping Brain, BIS/Brain Research Institute, UCLA, Los Angeles, 1972, Volume 1, pp. 376-393.

Snyder, F.: Unresolved questions concerning the interrelation of sleep loss and depression. In Proceedings of Symposium on Depression, sponsored by The Interdisciplinary Society of Biological Psychiatry of Holland, Amsterdam, The Netherlands, January 1974, in press.

Gillin, J.C., Buchsbaum, M.S., Jacobs, L.S., Fram, D.H., Williams, Jr., R.B., Vaughan, Jr., T.B., Mellon, E., Snyder, F. and Wyatt, R.J.: Partial REM sleep deprivation, Schizophrenia, and Field Articulation. Arch. Gen. Psychiat., in press.

Serial No. M-CP(C)-18-6
1. Laboratory of Clinical Psychobiology
2. Section on Comparative Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Comparative Studies of Sleep

Previous Serial Number: Same

Principal Investigator: Frederick Snyder, M.D.

Other Investigators: Nellie M. Bugbee, M.A. and Thomas C. Douthitt, Ph.D.

Cooperating Units: Section on Technical Development, DIRP
Department of Zoology, University of Nairobi and
Natural Museum of Kenya, Nairobi, Kenya

Man Years (1973):

Total:	1
Professional:	3/4
Other:	1/4

Project Description:

Objectives:

The general objective of this project is to explore the phylogenetic manifestations and adaptive functions of sleep by systematic comparative study in a broad variety of animal forms under varied experimental conditions. During the past year that goal was furthered by the principal investigator's foreign work assignment as a Visiting Professor at the University of Nairobi Medical School in Kenya, where it was possible to carry out studies of a number of mammalian species of particular phylogenetic interest. In addition, the assignment in Kenya offered opportunity for field trials of telemetric methods developed here for studies of sleep patterns in entirely free-roaming animals.

Methods Employed:

As in earlier studies done here, the Kenyan work involved recording of 24-hour sleep-waking patterns in animal subjects via implanted telemetry transmitters. In this case the animals had been acclimated to conditions of captivity in large (24-ft diameter) outdoor enclosures for at least several months prior to study. Individual animals to be studied, singly

or in colonies, depending upon species, were implanted with transmitters and allowed at least 10 days post-surgical recovery time before data collection. Without further disturbance to the animals, records were then obtained over at least 3-4 total 24-hour periods for each animal. Records are scored in 30-second epochs in terms of waking, transitional sleep, slow-wave sleep and REM sleep, as well as intervals of feeding defined by characteristic artifacts.

Attempted field studies of free-roaming animals employed a telemetry system designed by Mr. James Bryan, formerly Chief, Section on Technical Development, DIRP, which made use of a short-range implanted telemetry package together with a much higher powered "booster" transmitter attached to the animals by a neck collar. The procedure involved capture of selected animals by Cap-chur gun darting and chemical immobilization (Immobilon), surgical implantation of electrodes and transmitter in the field wherever the animal fell, and attachment of neck-collar containing "booster" transmitter. A timing device built into the system then turned off the transmitter power for a period of 6 days to permit recovery from surgery and the trauma of capture, after which the animal was relocated by signal from an incorporated "tracking" transmitter. By means of a high gain directional antenna and a mobile recording station housed in a Land-Rover, attempts were then made to record continuous electroencephalographic patterns of sleep and waking over 24-hour sampling periods.

Major Findings:

A. Studies of Small Captive Mammals

Studies involving at least 3-4 complete 24-hour records on 4 or more specimens were carried out on 7 species of small African mammals as follows:

(1) and (2) - Two species of hyrax, Heterohyrax brucei and Procavia Johnstoni, small rodent-like animals considered to exemplify a primitive stage of ungulate evolution.

(3) and (4) - Two species of prosimian primates, Galago crassicaudatus and Perodicticus potto, examples of the early lemuroid stage of primate evolution.

(5) and (6) - Two species of viverrid carnivores, Genetta genetta (common genet) and Herpestes sanguineus (slender mongoose),

(7) - The rodent, Cricetomys gambianus, the largest of true rats, of interest because of its size (up to 2 kg) in comparison to the much studied laboratory rat.

In addition, exploratory studies consisting of 4-day samples were obtained from single specimens of the following species: Dendrohyrax validus (tree hyrax); Ichneumia albicauda (white tailed mongoose); and Sylvicapra grimmia (bush duiker). Studies were also attempted on a species of elephant shrew, Petrodromus tetradactylus, of special interest in relation to our previous studies of other insectivores: Erinaceus europaeus (hedgehog) and Centetes ecaudatus (tenrec). A small colony of Petrodromus was successfully maintained in captivity for over 8 months, but efforts to implant them with telemetry transmitters were invariably fatal.

Of the 7 species from which complete studies were obtained, scoring of records and analysis of data has been completed for 4 (2 hyrax species, giant rat and genet) but scoring of records and analysis of data remains to be completed for the rest.

Findings will be exemplified by those concerned with hyrax, a group of animals frequently mentioned in the Old Testament, but still very little is known from the standpoint of behavior. Rock hyrax (Heterohyrax and Procavia) have always been described as diurnal, and their most notable behavioral feature has been their inactivity--authorities have been puzzled that they spent so much of the day resting and so little time feeding. Present results cast the natural history of hyrax in a different light. Records revealed that although both species do spend an average of 48% of every day in sleep, that sleep is distributed in short snatches throughout the 24-hours interspersed with periods of feeding. Although Heterohyrax spent as much time feeding at night as during the day, sleep was more prominent at night. For Procavia night-feeding predominated by far over day-feeding, and there were no significant differences in proportion of sleep between night and day. Sleep-waking proportions and polycyclic patterns of sleep-waking and feeding through 24-hours appear similar to those of many other small vegetarian mammals so far studied, such as Aplodontia, but relative scarcity of paradoxical sleep (5% of total sleep in Procavia, 8% in Heterohyrax) as well as special prominence of transitional sleep (52% and 40% of total sleep, respectively) are similar to findings in more progressive ungulates.

B. Field Trials on Free-Roaming Animals

Efforts to study 24-hour sleep-waking patterns of free-roaming animals in their natural habitats by means of long-range telemetry, something not yet accomplished for any species, were encouraging yet ultimately unsuccessful with the equipment and time available. Animals used for these trials were wild specimens of common zebra, Equus burchelli, chosen on the basis of their size and availability within a suitable

situation on a large private ranch about 70 miles south of Nairobi. Over the course of the year, 4 adult animals were captured, implanted with telemetry transmitters and recorded intermittently for varying durations. The first 2 attempts were failures due to problems in mechanical attachment of collars to the animals. No satisfactory recordings were obtained. After improvements in the design and attachments of collars good recordings were made from the last 2 animals for intervals up to 18 hours. Despite repeated and exhausting efforts, however, it was never possible to obtain complete 24-hour records.

Under field conditions in the bush, the range of our transmitters turned out to be considerably less than the 1/2 mile we had hoped for, and the mobility of our receiving station was severely restricted in darkness due to the hazards of hidden stumps, tree trunks and aardvark holes. Since the zebra were especially active at night, they always managed to elude our efforts to track them at some point during the hours of darkness.

Although these trials might have been much more successful with a different choice of species or a more favorable terrain, an effective telemetry range of at least 1/2 to 3/4 mile probably would be a minimal prerequisite to justify the considerable effort involved.

Significance to Mental Health Research:

By carefully examining the manifestations of sleep in different animal forms of diverse life adaptations, the work described strives to contribute toward a general biological understanding of the significance of sleep. We cannot hope to fathom the role of sleep and its disorders in human life without such mere basic understanding.

Proposed Course of the Project:

Due to the disbanding of this Laboratory no new studies in this area are contemplated. Efforts will continue to complete the processing of data already collected and to see the results through to publication.

Publications:

Snyder, F.: Evolutionary theories of sleep: What, which, and whither? In Chase, M.H. (ed.): The Sleeping Brain, BIS/Brain Research Institute, UCLA, Los Angeles, 1972, Volume 1, pp. 40-43.

Honors and Awards: None

Serial No. M-CP(C)-18-7
1. Laboratory of Clinical Psychobiology
2. Section on Comparative Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Predatory Aggression in the Rat

Previous Serial No.: Same

Principal Investigator: Thomas C. Douthitt, Ph.D.

Other Investigator(s): Jorge Perez-Cruet, M.D.

Cooperating Units: Laboratory of Clinical Science, DCBR

Man Years (1973): None

This project has been completed.

Major Findings:

Predatory aggression, as exemplified by mouse-killing in rats, has been shown to be mediated by a complex interaction of several neurochemical transmission systems. While utilizing a pharmacological approach to manipulate the mechanism responsible for mouse-killing by producing the behavior with a variety of drugs, this study also attempted to uncover the various factors influencing this behavior in animals who kill spontaneously. In spontaneous killers, it has been found that there is considerable diurnal influence which is primarily a product of light-dark cycles. Mouse-killing is potentiated by darkness and even in constant light reaches its peak during the normal night hours. Spontaneous killers had increased monoamine synthesis rates, but showed no differences from non-killers in terms of residual levels of serotonin, dopamine, or norepinephrine.

Depletion of serotonin levels consequent to tryptaminergic nerve terminal destruction with 5-6 dihydroxytyptamine was associated with the development of killing behavior in previously non-aggressive animals. This effect was enhanced by concurrent noradrenergic terminal destruction with 6-hydroxydopamine. The ability of cholinergic blockade with atropine to suppress killing in animals depleted both of serotonin and of catechol-

amines, but not in animals depleted only of serotonin led to the conclusion that both catecholaminergic and cholinergic mechanisms can support predatory aggression in preparations where the inhibitory influences of serotonin have been reduced.

Proposed Course of the Project:

The findings of this study are being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. M-CP(C)-18-8
1. Laboratory of Clinical Psychobiology
2. Section on Comparative Studies
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pharmacological Studies of Responsivity and Arousal Level in Adult and Neonatal Rats

Previous Serial Number: Same

Principal Investigator: Thomas C. Douthitt, Ph.D.

Other Investigators: Yvonne Brackbill, Ph.D. and Jorge Perez-Cruet, M.D.

Cooperating Units: Department of Obstetrics and Gynecology, Georgetown University, School of Medicine and Laboratory of Clinical Science, DCBR

Man Years (1973): None

This project has been completed.

Major Findings:

The early ontogeny of response to continuous white noise stimulation was studied in rats treated intra-cerebrally with 6-hydroxydopamine (6-OHDA) 24 hours after birth to determine the role of central noradrenergic mechanisms in the mediation of response. Heart rate and motility were recorded polygraphically during exposure to the stimulus and during a control period with no stimulation. Testing was carried out according to a cross-sectional design at 7, 14, and 28 days post-partum. Although assay of brain norepinephrine indicated significant depletions at all three ages (down by 85%), response measures did not reflect a difference between drug-treated and control animals until 28 days when 6-OHDA treated rats showed a depression of arousal during white noise while the controls showed increases in both heart rate and gross motor activity. The results indicate that an intact central noradrenergic system is necessary for the mediation of increased arousal during stimulation, an effect which becomes apparent only in later stages of development when adult levels of norepinephrine are approached.

Publications: Festschrift in honor of Dr. Sidney Bijou, in press, 1974

Serial No. M-CS-OC(C)-16
1. Laboratory of Clinical Science
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of pinched-off nerve endings (synaptosomes) as a model system for investigating the transport, binding and metabolism of biogenic amines in the central nervous system.

Previous Serial Number: Same

Principal Investigator: Robert W. Colburn

Other Investigators: Irwin J. Kopin, Nguyen B. Thoa (Guest Worker), Gertrude D. Maengwyn-Davies (Guest Worker), Jonathan Moss (Guest Worker), L.K.Y. Ng (Guest Worker), and K. S. Rajan.

Cooperating Units: Section on Experimental Therapeutics, LCS and Illinois Institute of Technology Research (Chicago).

Man Years:

Total	1.30
Professional	1.20
Other	.10

Project Description:

Objectives: Synaptosomes isolated from whole brain or specific brain regions represent an in vitro system similar in many respects to that present in vivo. Current basic problems being investigated are: (1) How do neurotransmitters pass through membranes? (2) How are they bound within vesicles? (3) What is the mechanism for their release? A concurrent study involves investigation of the effects of psychotomimetic drugs on the above parameters for neurotransmitter substances.

Methods Employed: Drugs used for treatment of depression or mania such as desmethylimipramine, lithium or narcotics are added to the synaptosome system. The uptake, binding and release of a variety of neurotransmitter substances are measured with or without drug given in vivo prior to isolation of the synaptosomes. Synaptosomes are fractionated into plasma membranes, synaptic vesicles and receptor proteins. The chelation chemistry of neurotransmitters is in addition being studied as a possible mechanism for binding and release in synaptosomes.

Major Findings: The synaptosomes accumulate, bind and release monoamines such as norepinephrine and serotonin much as they function in in vivo or in perfusion experiments. As in the peripheral sympathetic nervous system, the route of norepinephrine metabolism varies with the mode of its release. Uptake studies with neurotransmitters are of importance since reuptake is a major means for termination of chemically transmitted signals. Recent findings with the in vitro synaptosome system provide evidence that the addition of certain toxins simulate electrical stimulation even in such isolated preparations. The subcellular localization of drugs such as marijuana, morphine and diphenylhydantoin and their effects on the binding and release of neurotransmitters have been determined. At the chemical level, current studies involve the coordination of neurotransmitters to metal ions and metal-ATP and phospholipid complexes.

Significance to Biomedical Research and the Program of the Institute: The drugs altering monoamine function and metabolism have proved very useful in the modifying mood. The present studies provide information on a molecular basis for the action of such drugs.

Proposed Course of Project: With the above system, large numbers of very similar samples can be prepared and will be used in comparing a wide variety of drugs using kinetic methods to examine active uptake mechanisms with parameters such as binding constants and Vmax. Synaptosomes will be further fractionated to isolate carrier or receptor proteins.

Honors and Awards: None

Publications:

Colburn, R.W., Ng, Lorenz K., Lemberger, L., Kopin, I.J.: Subcellular distribution of Δ^9-tetrahydrocannabinol in rat brain. Biochem. Pharmacol. 23: 873-877, 1974.

Moss, J., Colburn, R.W., and Kopin, I.J.: Scorpion toxin-induced catecholamine release from synaptosomes. J. Neurochem. 22: 217-221, 1974.

Rajan, K.S., Colburn, R.W., Davis, J.M.: Metal chelates in the storage and transport of neurotransmitters: Interactions of Cu^{2+} with ATP and biogenic amines. J. Neurochem. 22: 137-147, 1974.

Serial No. M-CS-OC(C)-19
1. Laboratory of Clinical Science
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Metabolism, distribution, and biochemical effects of psychoactive drugs

Previous Serial Number: Same

Principal Investigator: Irwin J. Kopin and Julius Axelrod

Other Investigators: Robert W. Colburn, Richard Hawks (Guest Worker), Fredrik Foppen (Guest Worker), Lorenz K.Y. Ng and Nguyen B. Thoa (Guest Worker)

Cooperating Units: None

Man Years:

Total	2.5
Professional	1.5
Other	1.0

Project Description:

Methods Employed: Drugs are administered to animals and using mass spectroscopic, spectrofluorimetric or radio-immunological techniques, the drug and its metabolites are assayed in tissues or leady fluids (blood, C.S.F., urine). Activities of the metabolites are assessed with relation to uptake and release of animals.

Major Findings: Cocaine and its metabolites enter brain readily and can be detected in the C.S.F. The demethylated metabolite of cocaine is as active as the parent drug in inhibiting uptake of amines into synaptosomes, but other metabolites are inactive.

Δ^9 Tetrohydrocannobinol readily binds to synaptosomes, presumably because of its lipid solubility.

In rats, morphine withdrawal results in elevated plasma catecholamine levels. Both the behavioral effects and catecholamine response appear to be nearly completely blocked by electroacupuncture.

Significance to Biomedical Research and the Program of the Institute: Drug abuse is the result of both social factors and pharmacological effects of the drugs. Identification of the mode of action of such drugs and procedures which prevent their action or the effects of their withdrawal is a central issue to a rational approach to control of use of these agents.

Honors and Awards: None

Publications:

Ng, L.K.Y., Lamprecht, F., Williams, R.B. and Kopin, I.J.: Δ^9-Tetrahydrocannabinol and ethanol: Differential effects on sympathetic activity in differing environmental setting. Science 180: 1368-1369, 1973.

Ng, L.K.Y., Marsden, H.M., Colburn, R.W., and Thoa, N.B.: Population density and social pathology in mice. Differences in catecholamine metabolism associated with differences in behavior. Brain Res. 59: 323-330, 1973.

Serial No. M-CS-OC(C)-21
1. Laboratory of Clinical Science
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Study of Cerebral Catecholamine Metabolism in Primates and Man

Previous Serial Number: None

Principal Investigators: Michael Ebert and Irwin J. Kopin

Other Investigators: W. W. Havens, C. Popper and E. K. Gordon

Cooperating Units: Laboratory of Psychology, Unit on Clinical Pharmacology, Section on Experimental Therapeutics, Section on Psychiatry.

Man Years:		
	Total	3.0
	Professional	2.0
	Other	1.0

Project Description:

Objectives: To study metabolites of the catecholamines in C.S.F., blood and urine and design methods which may be applicable to study of amine metabolism in the brain of patients with various mental or neurological disorders and to examine the effects of drugs on these substances.

Methods Employed: Fluorometric, gas chromatographic and mass spectroscopic methods for assay of endogenous metabolites. Radioisotopes are used to study kenetics and distribution of the metabolites.

Major Findings: A very sensitive and highly specific method for simultaneous assay of homovanillic and HVA (metabolite of dopamine), vanillylmandelic acid (VMA) and 3-methoxy, 4-hydroxyphenylglycol (MHPG) (metabolites of norepinephrine) in body fluids has been developed.

Probenecid-blockade of acid transport produces striking increases in blood and CSF levels of HVA, but VMA is elevated only in the blood. This supports the view that MHPG and not VMA is the major metabolite of norepinephrine in the CSF.

Administration of deuterium labelled methionine results in the rapid appearance of labelled HVA and MHPG in the spinal fluid of monkeys. When the method for determination of the specific activity of the precursor, methionine, is perfected, kinetic studies can be developed to provide a measurement for the rate of formation of the O-methylated metabolites.

Significance to Biomedical Research and Programs of the Institute: Assessing the rate of metabolite formation in animals and patients provides information on the rate of amine utilization. These measurements should allow testing of hypothesis regarding the role of amines in neurological and psychiatric disorders and assessing the effects of drugs on the amines in brain.

Proposed Course of Project: Extension of the techniques, determining their validity in animals and their feasibility in Man.

Honors and Awards: None

Publications:

Gordon, E.K., Oliver, J., Black, K. and Kopin, I.J.: Simultaneous assay by mass fragmentography of vanillyl mandelic acid, homovanillic acid and 3-methoxy-4-hydroxy-phenethylene glycol in cerebrospinal fluid and urine. Biochemical Medicine (in press), 1974.

Serial No. M-CS-Ph-5
1. Laboratory of Clinical Science
2. Section of Pharmacology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemistry and pharmacology of the adrenergic nervous system.

Previous Serial Number: Same

Principal Investigators: Roland Ciaranello, Ronald Holz, G. Frederick Wooten

Other Investigators: Julius Axelrod

Man Years:

Total	4.0
Professional	3.0
Other	1.0

Project Description:

Objectives: To examine the neuronal and hormonal mechanisms that regulate catecholamine biosynthetic enzymes. To study genetic factors involved in the regulation of catecholamine biosynthetic enzymes and to study neurotransmitter release mechanisms.

Methods Employed: Biochemical, pharmacological, immunological and genetic techniques.

Major Findings: Mouse sublines (Balb) raised in separate laboratories over 40 generations showed differences in the catecholamine biosynthetic enzymes tyrosine hydroxylase, dopamine-ß-hydroxylase and phenylethylamine-N-methyltransferase. Each of these enzymes appear to be carried on a single gene but the three enzymes appear to be regulated by other genes. There is a genetically linked fighting behavior. Sublines of mice that have high catecholamine biosynthetic activity are more aggressive. Hypophysectomy increases the degradation of dopamine-ß-hydroxylase. Dopamine-ß-hydroxylase was found to be present in extraneural tissue.

The ionophores X 537A and A 23187 release dopamine from brain synaptosomes of the corpus striatum by different mechanisms. A 23187 releases by a Ca^{++} dependent exocytosis while X 537A releases dopamine from an intrasynaptosomal storage vesicle.

Significance to Biomedical Research and the Program of the Institute: Control of adrenergic mechanisms are central to the understanding of behavior and psychoactive drugs.

Proposed Course of Project: Regulation of adrenergic post-synaptic cells will be studied.

Honors and Awards: Dr. Axelrod received the Sollmann Award of the American Pharmacological Society; elected to the Scientific Advisory Board of the National Foundation Center for Biomedical Education; City College; Brookhaven Laboratory; M.I.T. (Dept. of Nutrition) and Jackson Laboratory.

Publications:

Axelrod, J.: The fate of noradrenaline in the sympathetic neurone. In: The Harvey Lectures, Series 67, Academic Press, New York, 1973, pp. 175-197.

Ciaranello, R.D. and Axelrod, J.: Genetically controlled alterations in the rate of degradation of phenylethanolamine N-methyltransferase. J. Biol. Chem. 248: 5616-5623, 1973.

Coyle, J.T. and Henry, D.: Catecholamines in fetal and newborn rat brain. J. Neurochem. 21: 61-67, 1973.

Coyle, J.T., Jacobowitz, D., Klein, D., and Axelrod, J.: Dopaminergic neurons in explants of substantia nigra in culture. J. Neurobiol. 4: 461-470, 1973.

Coyle, J.T., Wender, P., and Lipsky, A.: Avoidance conditioning in different strains of rats: Neurochemical correlates. Psychopharmacologia (Berl.) 31: 25-34, 1973.

Matta, R.J. and Wooten, G.F.: Pharmacology of fusaric acid in man. Clin. Pharmacol. Ther. 14: 541-546, 1973.

Coyle, J.T., Jr.: Development of the central catecholaminergic neurons. In: Schmitt, F.O. and Worden, F.G. (eds.): The Neuroscience Third Study Program, Cambridge, Mass., M.I.T. Press, 1974, pp. 877-884.

Axelrod, J.: Regulation of the neurotransmitter noradrenaline. In: Schmitt, F.O. and Worden, F.G. (eds.): The Neuroscience Third Study Program, Cambridge, Mass., M.I.T. Press, 1974, pp. 863-876.

Coyle, J.T. and Kuhar, M.J.: Subcellular localization of dopamine-ß-hydroxylase and endogenous norepinephrine in the rat hypothalamus. Brain Research 65: 475-487, 1974.

Serial No. M-CS-Ph-6
1. Laboratory of Clinical Science
2. Section on Pharmacology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biogenic amines and psychoactive drugs.

Previous Serial Number: Same

Principal Investigators: Julius Axelrod, Michael Brownstein
Miklos Palkovits (Guest Worker) and
Juan Saavedra (Visiting Scientist)

Other Investigators: David Carpenter, Dept. of Neurobiology, Armed Forces Radiobiology Research Institute, NMC; Robert Utiger, Dept. of Medicine, Univ. of Pa.; Akira Arimura, Endocrinology Section, Veterans Administration Hospital, New Orleans, La.; and Wylie Vale, Salk Institute, La Jolla, Calif.

Man Years:		
	Total	4.5
	Professional	3.5
	Other	1.0

Project Description:

Objectives: To develop micromethods for the measurement of normally occurring biogenic amines. To localize these amines and releasing factors in nuclei of brain and identifiable nerve cell bodies in aplysia.

Methods Employed: Biochemical, pharmacological, enzymatic and radiochemical techniques.

Major Findings: Using sensitive enzymatic assays and microdissection procedures which can reproducibly remove 130 identifiable nuclei in the rat brain, the distribution of serotonin, noradrenaline, dopamine, histamine and tyrosine hydroxylase was described. Octopaminergic neurones were demonstrated in cells of aplysia. Several putative neurotransmitters were found to coexist in single identifiable neurones of the aplysia. Thyroxin releasing factor was found to be present in nuclei of the hypothalamus as well as other brain areas. Leutinizing hormone releasing factor was found only in the arcuate nucleus. Phenylalanine and MAO inhibitors were found to greatly elevate brain phenylethylamine.

Significance to Biomedical Research and the Program of the Institute: The localization of biogenic amines and their enzymes in specific nuclei of the rat brain will enable investigators to study the effects of drugs and physiological and behavioral manipulations on biogenic amines in a more precise manner. The coexistance of several neurotransmitters in single identifiable neurones should change our concepts on the nature of neurotransmission.

Proposed Course of Project: The effect of endocrinological, pharmacological, physiological and behavioral manipulations on levels and turnover of biogenic amines in specific brain nuclei will be examined.

Honors and Awards: Dr. Axelrod, Visiting Scholar Herbert Lehman College, City College, N.Y.; Visiting Lecturer: University of Colorado, Texas, Kansas, West Virginia, Medical College of Pennsylvania, Georgetown. Dr. Saavedra served as a WHO Fellow in Buenos Aires, Argentina and invited to give papers in symposium in Sardinia and Strasbourg.

Publications:

Coyle, J.T., Jacobowitz, D., Klein, D., and Axelrod, J.: Dopaminergic neurons in explants of substantia nigra in culture. J. Neurobiol. 4: 461-470, 1973.

Saavedra, J.M., Brownstein, M., and Axelrod, J.: A specific and sensitive enzymatic-isotopic microassay for serotonin in tissues. J. Pharmacol. Exp. Ther. 186: 508-515, 1973.

Saavedra, J.M., Coyle, J.T., and Axelrod, J.: The distribution and properties of the nonspecific N-methyltransferase in brain. J. Neurochem. 20: 743-752, 1973.

Saavedra, J.M. and Axelrod, J.: The normal occurrence of tryptamine in brain and its conversion to N-methyl and N-dimethyltryptamine in vitro and in vivo. In: Barchas, J. and Usdin, E. (eds.): Serotonin and Behavior. Academic Press, New York, 1973, pp. 129-137.

Saavedra, J.M. and Axelrod, J.: Effect of drugs on the tryptamine content of rat tissues. J. Pharmacol. Exp. Ther. 185: 523-529, 1973.

Wyatt, R.J., Saavedra, J.M., Belmaker, R., Cohen, S., and Pollin, W.: The dimethyltryptamine-forming enzyme in blood platelets: A study in monozygotic twins discordant for schizophrenia. Am. J. Psychiatry 130: 1359-1361, 1973.

Wyatt, R.J., Saavedra, J.M., and Axelrod, J.: A dimethyltryptamine-forming enzyme in human blood. Am. J. Psychiat. 130: 754-760, 1973.

Axelrod, J.: The Sollmann Oration. The Pharmacologist 16: 11-15, 1974.

Axelrod, J. and Saavedra, J.M.: Octopamine, phenylethanolamine, phenylethylamine and tryptamine in the brain. In: Aromatic Amino Acids in the Brain, Ciba Foundation Symposium, 22 (new series). ASP, Amsterdam, 1974, pp. 51-60.

Kreuz, D.S. and Axelrod, J.: Amphetamine in human plasma: A sensitive and specific enzymatic assay. Science 183: 420-421, 1974.

Saavedra, Juan M.: Enzymatic-isotopic assay for and presence of ß-phenylethylamine in brain. J. Neurochem. 22: 211-216, 1974.

Serial No. M-CS-Ph-7
1. Laboratory of Clinical Science
2. Section on Pharmacology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical and pharmacological studies on the pineal gland.

Previous Serial Number: Same

Principal Investigator: Jorge Romero

Other Investigators: Julius Axelrod, Michael Brownstein and Ronald Holz

Man Years:

Total	3.0
Professional	2.0
Other	1.0

Project Description:

Objectives: To use the pineal as a model to learn how nerves interact with responsive cells.

Methods Employed: Biochemical pharmacological and radioactive tracer techniques.

Major Findings: A circadian rhythm in responsiveness in the ability to synthesize serotonin N-acetyltransferase in the pineal has been observed. The pineal is 10 times more sensitive during the daytime when the release of neurotransmitter is reduced than during the nighttime when transmitter release is increased. The daily rhythm in supersensitivity in the postjunctional pineal cell and turnover of noradrenaline in prejunctional nerves provides amplification and dampening mechanisms for response of postjunctional cells. Drugs such as cocaine, monoamine oxidase inhibitors, veratridine and high potassium stimulate the synthesis of pineal N-acetyltransferase by increasing the amount of catecholamine neurotransmitter reaching the pineal.

Significance to Biomedical Research and the Program of the Institute: The problems of nerve cell interactions and circadian rhythm are of prime importance to neurobiology and behavior. The pineal has served as a useful model to study these phenomena.

Proposed Course of Project: Further studies of mechanisms as to how neurotransmitters change cell responsiveness will be explored.

Honors and Awards: None

Publications:

Brownstein, M., Holz, R.W., and Axelrod, J.: The regulation of pineal serotonin by a ß-adrenergic receptor. J. Pharmacol. Exp. Ther. 186: 109-113, 1973.

Brownstein, M., Saavedra, J.M., and Axelrod, J.: Control of pineal N-acetylserotonin by a Beta adrenergic receptor. Mol. Pharmacol. 9: 605-611, 1973.

Deguchi, T.: Role of the Beta adrenergic receptor in the elevation of adenosine cyclic 3', 5'-monophosphate and induction of serotonin N-acetyltransferase in rat pineal glands. Mol. Pharmacol. 9: 184-190, 1973.

Deguchi, T. and Axelrod, J.: Supersensitivity and subsensitivity of the ß-adrenergic receptor in pineal gland regulated by catecholamine transmitter. Proc. Nat. Acad. Sci. 70: 2411-2414, 1973.

Deguchi, T. and Axelrod, J.: Superinduction of serotonin N-acetyltransferase and supersensitivity of adenyl cyclase to catecholamines in denervated pineal gland. Mol. Pharmacol. 9: 612-618, 1973.

Brownstein, M. and Axelrod, J.: Pineal Gland: 24-hour rhythm in norepinephrine turnover. Science 184: 163-165, 1974.

Holz, R.W., Deguchi, T., and Axelrod, J.: Stimulation of serotonin N-acetyltransferase in pineal organ culture by drugs. J. Neurochem. 22: 205-209, 1974.

Serial No. M-CS-Ph-8
1. Laboratory of Clinical Science
2. Section on Pharmacology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on protein carboxymethyltransferase.

Previous Serial Number: None

Principal Investigators: Emanuel Diliberto, Julius Axelrod

Man Years:

Total	1.5
Professional	1.5
Other	0

Project Description:

Objectives: To study the properties, distribution and physiological disposition of protein carboxymethyltransferase.

Methods Employed: Enzymatic and radiometric.

Major Findings: The methanol-forming enzyme described in our laboratory in 1965, methylates peptide hormones LH, growth hormone, TRF, ACTH in the pituitary. The enzyme has the highest localization in the brain.

Significance to Biomedical Research and the Program of the Institute: Not certain yet.

Proposed Course of Project: The physiological significance of this enzyme will be explored.

Honors and Awards: None

Publications:

One in press.

Serial No. M-CS-M(C)-08
1. Laboratory of Clinical Science
2. Section on Medicine
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Formation, release, disposition and metabolism of biogenic amines

Previous Serial Number: Same

Principal Investigator: Irwin J. Kopin

Other Investigators: Robert Colburn, Ingeborg Hanbauer, J. Stephen Kizer, Ronald Kobayashi, Antonia Liuzzi, Jonathan Moss (Guest Worker), John L. Reid, Nguyen B. Thoa (Guest Worker), Michael Roizen, James Thomas, Virginia Weise, and Michael Ziegler.

Cooperating Units: Sections on Pharmacology, LCS, and Histopharmacology, LCS.

Man Years:

Total	8.5
Professional	1.5
Other	7.0

Project Description:

Objectives: (1) To examine the mechanisms which control synthesis, storage, release, action and termination of action of amine neurotransmitters. (2) To determine how drugs interact with these mechanisms. (3) To define how genetic or environmental influences affect these control mechanisms.

Methods Employed: Radioactive amines or their precursors are used to study the formation and disposition of the endogenous amines, the amines and their biosynthetic enzymes are assayed radiometrically or localized by histofluorescent techniques, and the effects of drugs, toxins, hormonal states, or stresses examined.

Major Findings: Dopamine-β-hydroxylase, which is released along with norepinephrine, is useful as a marker of exocytosis. Drugs, such as veratridine, or high levels of potassium, cause depolarization of the neuronal membrane and release of norepinephrine by exocytosis while other agents, such as sympathomimetic amines, release the neurotransmitter by displacement of the amine

and exocytosis is not involved. Calcium ions are usually necessary for exocytosis and ionophores which transport Ca++ across the neuronal membrane produce exocytotic release of the transmitter. Scorpion venom contains a specific toxin which causes release of norepinephrine by an exocytotic mechanism by an action on the cell membrane which mimics stimulation of the nerve.

Dopamine-β-hydroxylase in the plasma may be transiently elevated during procedures which result in sympathetic neuronal activity, but over a prolonged period of time the plasma enzyme levels are remarkably constant. Even striking changes in the plasma norepinephrine content may not be accompanied by alterations in the levels of this enzyme. The plasma levels of DBH, are elevated when the synthesis rate of the enzyme is increased (e.g., by administration of reserpine) or with changes in hormones (e.g., hypothyroidism). The enzyme is synthesized in the sympathetic nerve cell bodies and is transported to the nerve ending where there appears to be a non-exocytotic mechanism for continuous release of the enzyme as well as the stimulation-induced exocytotic mechanism. Genetic as well as hormonal and neural factors influence plasma levels of dopamine-β-hydroxylase.

In the rat, most of the circulating catecholamine is epinephrine. Plasma levels of this amine almost disappear after adrenalectomy but norepinephrine levels are only slightly altered. During anesthesia with halothane levels of plasma epinephrine and norepinephrine are markedly depressed both by central and direct peripheral effects of the anesthetic agent. There is much less catecholamine in blood from the tail vein of a rat than in blood obtained from the arteries. This difference appears to be due to both uptake by sympathetic nerves and metabolism of the amine. The levels of catecholamines in the venous blood may vary with nerve activity but the levels of dopamine-β-hydroxylase are similar in venous and arterial blood.

Vinblastine, which disaggregates microtubules, diminishes the rate of norepinephrine release and after one day results in decreased norepinephrine levels in the heart. There is a reversible inhibition of norepinephrine uptake, but with chronic treatment there is destruction of the nerve endings similar to that seen after 6-hydroxydopamine administration.

Tyrosine hydroxylase, the enzyme which is rate-limiting in norepinephrine synthesis, is increased when amine release is enhanced for any significant period of time. A transient rise in cyclic-AMP appears to be a necessary prerequisite to initiate the train of events which leads to the increased levels of this enzyme. Agents which cause a rise in cyclic AMP also produce induction of this enzyme. The levels of the enzyme have been measured in small areas of the rat brain and are elevated in specific nuclei with

changes in endocrine status or with stress. Stress does not, however, alter the turnover rate of histamine.

Lesions of the locus coeruleus result in alterations in levels of norepinephrine in many areas of the brain. Sensitive biochemical measures of the degree of change in catecholamine levels provides information about innervation of these areas from the cells in the locus coeruleus. The relatively narrow range over which fluorescence was found to change with alterations in catecholamine content explain why such changes were not apparent when the histofluorescent techniques were used.

Significance to Biomedical Research and the Program of the Institute: Biogenic amines play an important role in the physiology of brain and in the peripheral sympathetic nervous system. Alterations in the synthesis, release, metabolism, and action of these substances often explain how drugs or toxins act and have been thought to play a role in hormonal effects, drug addiction, and mental as well as neurological disorders.

Proposed Course: Study of the controls of snythesis, release, metabolism action and inactivation of biogenic amines in isolated tissues, animals and patients will be continued and attempts to define the effects of drugs, hormones and emotional states will be pursued.

Honors and Awards: Dr. Ronald Kobayashi was awarded the Weir-Mitchell award of the American Neurological Society for his work on catecholamines in brain. Dr. Irwin Kopin was invited to participate in symposia at the American Neurological Society meeting and acted as consultant to Health Learning Systems in developing a monograph on the "Catecholamines."

Publications:

Hanbauer, I.: Induction of tyrosine hydroxylase in superior cervical ganglion by cold exposure: Effects of decentralization and mecamylamine. In S. Snyder and E. Uzdin (Eds.): Frontiers in Catecholamine Research, Pergamon Press, London 1973.

Hanbauer, I., Jacobowitz, D.M. and Kopin, I.J.: Effects of vinblastine on noradrenergic axons. Brit. J. Pharmaccl. 50: 1974.

Hanbauer, I., Kopin, I.J. and Jacobowitz, D.M.: Effects of vinblastine on peripheral autonomic nerves: Degeneration and formation of new nerve fibers. Presented to Wenner-Gren Center International Symposium ("Dynamics of Degeneration and Growth in Neurons"), Stockholm, May 16-18, 1973.

Hanbauer, I., Kopin, I.J. and Costa, J.: Mechanisms involved in the trans-synaptic increase of tyrosine hydroxylase and DBH activity in sympathetic ganglia. Naunyn-Schmiedeberg's Arch. Pharmacol. 280: 39-48, 1973.

Kizer, J.S., Palkovits, M., Zivin, J., Brownstein, M., Saavedra, J. and Kopin, I.J.: The effect of endocrinological manipulations on tyrosine hydroxylase and dopamine-β-hydroxylase activities in individual hypothalamic nuclei of the adult male rat. Endocrinology (in press) 1974.

Kobayashi, R.: Biochemical mapping of the noradrenergic projection from the locus coeruleus: A model for studies of brain neuronal pathways. Submitted to the American Academy of Neurology, S. Weir Mitchell Award, 1974.

Kobayashi, R., Brownstein, M., Saavedra, J. and Palkovits, M.: Choline acetyltransferase content in discrete regions of the rat brain stem. J. Neurochem. (in press).

Kobayashi, R. and Kopin, I.J.: Effects of stress and environmental lighting on histamine in the rat brain. Brain Research (in press).

Kobayashi, R., Palkovits, M., Kopin, I.J. and Jacobowitz, D.: Biochemical mapping of noradrenergic nerves arising from the rat locus coeruleus. Brain Research (in press).

Kopin, I.J., Palkovits, M., Kobayashi, R. and Jacobowitz, D.: Quantitative relationship of catecholamine content and histofluorescence in brain of rats. Brain Research (in press).

Lamprecht, F., Wyatt, R.J., Belmaker, R., Murphy, D.L. and Pollin, W.: Plasma dopamine-β-hydroxylase in identical twins discordant for schizophrenia. In S. Snyder and E. Uzdin (eds.): Frontiers in Catecholamine Research, Pergamon Press, London 1973.

Moss, J., Colburn, R. and Kopin, I.J.: Scorpion toxin-induced catecholamine release from synaptosomes. J. Neurochem. (in press).

Moss, J., Thoa, N. and Kopin, I.J.: On the mechanism of scorpion toxin-induced release of norepinephrine from peripheral adrenergic neurons. J. Pharmacol. Exp. Ther. (in press).

Thoa, N.: Synthesis and preferential release of newly formed norepinephrine during increased sympathetic activity. In S. Snyder and E. Uzdin (eds.): Frontiers in Catecholamine Research, Pergamon Press, London 1973.

Thoa, N., Costa, J., Moss, J and Kopin, I.J.: Mechanism of release of norepinephrine from peripheral adrenergic neurones by the calcium ionophores X 537A and A 23187. Life Sciences (in press).

Thoa, N., Wooten, F., Axelrod, J. and Kopin, I.J.: Mechanism of release at noradrenergic nerve terminals by reserpine and several sympathomimetic agents. Molecular Pharmacol. (in press).

Thomas, J., Van Orden,L.S., Redick, J.A. and Kopin, I.J.: Immunological localization of dopamine-β-hydroxylase on the chromaffin granule membrane. In S. Snyder and E. Uzdin (eds.): Frontiers in Catecholamine Research, Pergamon Press, London 1973, p. 79-82.

Serial No. M-CS-M(C)-12
1. Laboratory of Clinical Science
2. Section on Medicine
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Growth characteristics of aminergic neurones

Previous Serial Number: Same

Principal Investigator: Irwin J. Kopin

Other Investigators: Ingeborg Hanbauer, Jonathan Moss, James Thomas, Jerry Webb, Virginia Weise, Michael Ziegler

Cooperating Units: None

Man Years:

Total	2.5
Professional	2.0
Other	0.5

Project Description:

Objectives: Develop in vitro and in vivo techniques to study factors which control processes of growth, development, and regeneration of neurones.

Methods Employed: Organ culture of ganglia or brain parts or transplantation of neuronal tissue, radiometric assay of enzymes, amines, and amine uptake.

Major Findings: Sympathetic ganglia in organ culture undergo many changes which are consequences of pre- and past-ganglionic nerve section. Prevention of axonal transport results in an accumulation of catecholamines at the post axonal stump which is due to synthesis and retention of the amine in storage vesicles. After two days in culture levels of the enzymes, tyrosine hydroxylase and dopamine-β-hydroxylase decrease, but dopamine synthesis is suddenly accelerated. The accumulation of dopamine occurs throughout the ganglion, not at the severed stumps and is presumed to be due to development of axonal sprouts by the small intensely fluorescent interneuronal dopaminergic cells in the ganglion. The synthesis rate of dopamine-β-hydroxylase, measured as immunoprecipitable radiolabelled enzyme, is enhanced in ganglia from reserpine treated rats.

Significance to Biomedical Research and Program of the Institute: Understanding factors controlling neuronal growth and development is essential to a rational approach to investigation of physiologic processes (e.g. memory, maturation, endocrine control) and pathological conditions (mental retardation, drug effects on the fetus, and genetic influences on behavior).

Proposed Course of Project: Tissues from fetal, infant and mature rat brain and peripheral sympathetic neurones will be examined in vitro for changes in enyzmes, transport processes, release mechanisms, and biochemical effects to determine effects of drugs and hormones on development.

Honors and Awards: None

Publications:

Kopin, I.J., Berv, K.R. and Webb, J.G.: Sympathetic ganglia in organ culture. In S. Snyder and E. Uzdin (Eds.): Frontiers in Catecholamine Research, Pergamon Press, London, 1973.

Serial No. M-CS-HP-1
1. Laboratory of Clinical Science
2. Section on Histopharmacology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Morphologic and functional studies of central and peripheral aminergic and cholinergic neurons

Previous Serial Number: M-CS-OC(C)-20

Principal Investigator: David M. Jacobowitz

Other Investigators: Miklos Palkovits, Paul D. MacLean, Lloyd A. Greene, Kenneth Kent, Theodore Cooper

Cooperating Units: Laboratory of Brain Evolution and Behavior, NIMH; Cardiology Branch, NHLI

Man Years:		
	Total	4.9
	Professional	2.0
	Other	2.9

Project Description:

Objectives: (1) A multidisciplinary approach to the study of the adrenergic nervous system is utilized with major emphasis on monoaminergic nerves in the brain. Localization of monoaminergic nerves (noradrenergic, dopaminergic, serotonergic) are accomplished first by histochemical means. Drugs which have the ability to selectively destroy noradrenergic or serotonergic nerves are administered to rats. Correlations of chemical and histochemical analyses of brain amines, monoaminergic neurons and behavioral observations are made. (2) Localization of cholinergic neurons in the brain by a histochemical method. This will provide the basis for the long-term objective for studying central adrenergic-cholinergic interactions. (3) Use of tissue culture to study nerve growth in organ and dissociated cell cultures. (4) On a long-term basis, this project represents an investigation of both the morphological basis for catecholaminergic mechanisms and the functional organization of the autonomic nervous system in several organs.

Methods Employed: (1) The morphological procedure involves the use of a specific fluorescence histochemical method for the demonstration of catecholamines (norepinephrine, epinephrine, dopamine) and indoleamines (serotonin) within monoaminergic nerves and cells. (2) Biochemical assays for norepinephrine, dopamine, and serotonin are available. (3) The histochemical procedure for cholinergic neurons by the use of the acetylcholinesterase-staining technique. (4) Brain stereotaxic method for intraventricular injections of drugs. (5) Microregional dissection of the brain into discrete brain nuclei and subdivisions followed by radioisotopic assay of norepinephrine.

(6) Immunofluorescence microscopy of dopamine-B-hydroxylase (DBH).
(7) Behavioral parameters such as water and food consumption, spontaneous motor activity, rectal temperature and emotional arousal are measured.

Major Findings: (1) We have previously shown that the intraventricular injection of 6-hydroxydopa (6-OHDOPA) causes a graded, dose-dependent reduction in brain norepinephrine (NE) without affecting brain dopamine (DA) levels. Emotionality ratings were increased by the injection of 6-OHDOPA and returned to normal only after 70 days when NE levels in the cortex and hindbrain had only returned to normal. More recently, we have studied the serotonergic nerve contribution to various behavioral changes. The intraventricular injection of the neurotoxic agents 6-OHDOPA and 5,6-dihydroxytryptamine, alone or in combination produces a specific reduction in brain NE and 5-HT, respectively, by the selective destruction of noradrenergic or serotonergic nerve terminals. 6-OHDOPA reduced consumatory and locomotor behaviors, and increases emotionality. The deficits in food and water consumption may be due to the reduction of brain norepinephrine levels which disrupts the balance between the noradrenergic and the dopaminergic neurotransmitter systems. The reduced open field locomotion is correlated with an absolute increase in brain serotonin levels that occurs during the few days just after the injection of 6-OHDOPA. The increased emotionality might be due to a relative predominance of 5-HT in the NE-5-HT balance that underlies emotional tone. (2) Catecholaminergic terminals and axonal pathways of the brain continue to be mapped. A complete stereotaxic neuroanatomical atlas of the rat brain was prepared using transverse serial sections stained with luxol fast blue and cresyl violet. Catecholamine-containing cell bodies and fiber terminals were identified by the histofluorescence method, whereas the AChE distribution was determined by the aid of Koelle and Friedenwald's histochemical method. AChE is the enzyme generally thought to be a good indicator of cholinergic neurons. A detailed stereotaxic atlas of the catecholaminergic and AChE-containing structures is in press. Such a mapping will point out regions of correlation between central adrenergic and cholinergic neurons. Furthermore, a more rational approach to the interpretation of a wide variety of experimental approaches to the study of the central nervous system by surgical manipulation or drug placement can be accomplished by the availability of a comparative atlas of catecholaminergic and AChE-staining brain structures.

Currently a similar localization of aminergic neurons and serotonergic cell bodies is being mapped in the monkey brain (with Dr. Paul MacLean). A remarkable similarity between the monkey and rat aminergic neurons is apparent.

The intraventricular injection of the neurotoxic agent 6-hydroxydopa (6-OHDOPA) has revealed localization of discrete pathways to individual regions of the brain (hippocampus, septum, cortex, amygdala, hypothalamus, thalamus). Stereotaxic placement of discrete lesions are currently being performed to study innervation patterns of the dorsal and ventral noradrenergic axonal pathways and the dopaminergic nigrostriatal routes of passage.

Lesion of the locus coeruleus correlated with biochemical assays (Henry method) of small nuclei of the brain (Palkovits method) has revealed that the para-

ventricular and periventricular nuclei and the habenulae of the diencephalon receive a noradrenergic contribution from the locus coeruleus. In addition a crossed innervation of noradrenergic nerves takes place at the posterior commissure to innervate the inferior colliculi and geniculate bodies. Further more, it appears that the cortical noradrenergic nerves are not solely derived from the locus coeruleus cell bodies. (3) The influence of gold-thioglucose, a drug capable of destroying the ventromedial nucleus of the mouse brain was shown to specifically deplete the median eminence of catecholamines without destruction of the dopaminergic cell bodies of the arcuate nucleus. An involvement of the median eminence-pituitary amines in the gold-thioglucose-induced obesity in mice is not known. Studies along these lines will be pursued. (4) A study was undertaken to investigate the origin of the ventral cholinergic pathway of the rat brain. Electrical or chemical (vinblastine) lesions resulted in a retrograde accumulation of AChE which could be followed caudally to the nucleus cuneiformis and the dorsolateral tegmental nucleus. (5) A unilateral injection of 6-OHDA in the region of the ventromedial nucleus of rat brains prevented the rise in blood pressure observed after DOCA pretreatment with unilateral nephrectomy. A unilateral buildup of CA fluorescence could be noted in the medial forebrain bundle on the side of the cannula placement. The enhanced fluorescence was contained in axons of the dopaminergic tracts within the median forebrain bundle. These tracts could be followed caudally to the level of the 6-OHDA injection to the mammilary body region just medial to the crus cerebri and nucleus subthalamicus. Of these animals, a prominent reduction in the number of dopaminergic cell bodies of the substantia nigra was noted on the side of the lesion. Therefore, the 6-OHDA destroyed a considerable number of dopaminergic axons within the median forebrain bundle resulting in a marked reduction of dopamine in the caudate-putamen. The involvement of the nigrostriatal and mesolimbic dopaminergic fibers will be pursued further. (6) Superior Cervical ganglion cells of newborn rats implanted within the lateral ventricles of adult rats survived and maintained catecholamine as revealed by fluorescence microscopy. The lateral ventricles may prove useful for "in vivo" culture of normal and malignant cells. (7) The utility of semi-quantitation of catecholamine in fluorescent sections of the rat brain was studied by correlating fluorescence intensity in aminergic nerves with catecholamine content assayed by a sensitive radiometric method for norepinephrine and dopamine. Reductions in amine content followed several doses of reserpine. The range over which fluorescence appeared to diminish in the paraventricular and dorsomedialis nucleus, the anterior ventral thalamic nucleus and cingulate cortex was only about 20-40% of the normal catecholamine content. The degree of catecholamine depletion as observed by fluorescence microscopy varies with the region examined. In areas with a high catecholamine content a greater reduction in amine content was required to make a fluorescence decrease noticeable than in areas with a normally lower catecholamine content. (8) The NE content of rat superior cervical ganglia grown in culture for two days increased four fold while the DA increased seven fold. After four days, the NE had declined to approximately 50% of its peak level while DA remained elevated near its maximum value. Histofluorescence studies revealed a marked increase in fluorescence of the postganglionic trunks in addition to a moderate increase in the number of intraganglionic varicose fibers. No changes were noted in the number of chromaffin cells within the ganglia.

Cultures maintained for two weeks showed a complete destruction of adrenergic cell bodies while chromaffin cells were still present. This correlated with small amounts of dopamine and essentially no NE. These studies support the fact that chromaffin cells of the superior cervical ganglion contain DA and suggest that dopamine-containing fibers sprout during the early stages of organ culture.

Immunofluorescence studies using the DBH antibody however, revealed that the chromaffin cells of the guinea pig coeliac ganglion contains DBH while that of the superior cervical ganglion did not. This indicates that the extra-adrenal chromaffin cell amine content probably varies depending on individual ganglion localization. This further supports our contention that ganglion chromaffin cells contain processes and thereby act as interneurons.

(9) Chromaffin cells of dissociated chick embryo sympathetic ganglia were studied by catecholamine fluorescence microscopy in long-term cell cultures. These cells were distinguishable in culture from the sympathetic ganglionic neurons by their small size, relative infrequency of occurrence, intense fluorescence and survival in the absence of nerve growth factor. By several weeks in culture, with or without nerve growth factor in the medium, the chromaffin cells produced extensive ramifications of fluorescent varicose processes. Chemical assay of these cells show that the amine present is norepinephrine/epinephrine rather than dopamine. It is suggested that at least part of the intraganglionic system of adrenergic varicose terminals arises from chromaffin cells. This capability with regard to fiber production lends credence to the suggestion that chromaffin cells may play an active role in ganglionic function. (10) Immunofluorescence of the salivary gland of rats after chronic removal of the superior cervical ganglion revealed the presence of deposits of dopamine-B-hydroxylase (DBH) specifically localized within the acinar lumen of the sublingual gland but not the submaxillary gland. The physiological significance of this observation is unknown. It is suggested that the sublingual gland is an important excretory organ for circulating DBH. (11) A study was undertaken to establish a minimal effective dose of 6-OHDA capable of producing and maintaining a sympathetic denervation of young dogs. This is important for long-term studies on the effects of painful or aversive stimuli upon classical conditioning, instrumental learning, physiological and behavioral development in the dog. Initial large i.v. or i.p. doses caused a sympathectomy that was maintained for at lease one week. After one month, partial reinnervation to the heart was observed. Long-term administration of 7 mg/kg caused a considerable reduction in the cardiac NE content. It was suggested that a chronic i.p. dose of approximately 7 mg/kg administered once every two weeks could maintain an adequate long-term cardiac sympathectomy. (12) Increased vagal tone and associated bradycardia occurring during acute myocardial infarction in man previously was thought to predispose to ventricular fibrillation (VF). However, during experimental acute myocardial infarction, increased vagal tone increases VF threshold (VFT) and reduced incidence of spontaneous VF. In this study, we have localized the anatomic pathways responsible for these effects. Vinblastine, a neurotoxic agent, was injected into the paraaortic area adjacent to the

AV node. This area contains ganglia that supply postganglionic cholinergic fibers to the ventricle. Five to seven days later, vagal stimulation in these dogs caused atrial slowing but did not raise VFT. Thus, vagal efferents to the atria were intact but those to the ventricle, responsible for increasing electrical stability, were inoperative. Histochemical analysis was performed to determine the anatomic correlate of this electrophysiologic finding. Atrial cholinergic nerves were intact; however, the cholinergic nerves that normally surround the Purkinje system was absent. Thus, enhanced ventricular electrical stability induced by vagal stimulation appears mediated by a network of cholinergic nerves intimately related to Purkinje fibers in the ventricular system. (13) A histochemical examination of catecholamine-containing elements was undertaken in the calf and dog lung. This study demonstrated that an intraganglionic system of varicose fibers are situated in cholinergic ganglia of the calf lung similar to that previously observed in cholinergic ganglia of the gut, heart and sympathetic ganglia. The ganglia were also found to contain a small number of chromaffin cell clusters similar to those previously observed in cholinergic ganglia of the heart and in sympathetic ganglia. The presence of catecholamine-containing varicosities in cholinergic ganglia of calf lung suggests that the sympathetic neurotransmitter has a physiologic role in ganglionic transmission.

Significance to Biomedical Research and the Program of the Institute: Our studies attempt to elucidate the functions of catechol- and other amines in the brain and periphery in normal and pathological clinical states. The use of specific neurotoxic drugs offers a technique with high resolution for investigation of the anatomical and functional organization of the norepinephrine neurons in the brain. Investigations which utilize the neurochemical specificity of the neurotoxic agents should contribute significantly to the understanding of the neural mechanisms controlling behavior.

Precise localization of monoaminergic tracts in the brain will enable researchers to make discrete lesions or administer specific neurotoxic agents to denervate small regions of the brain. Further behavioral testing will reveal the functional influences of the monoaminergic or cholinergic tracts in the brain.

Proposed Course of Project: Mapping of monoaminergic and cholinergic pathways of the brain will continue. Destruction of various tracts will be undertaken along with biochemical, behavioral and histopharmacological studies of the brain.

Honors and Awards: (1) Invited speaker to the "Gordon Research Conference on Catecholamines" in Wolfboro, New Hampshire (Aug. 12-17, 1973).
(2) Invited speaker to the Conference on "Neurobiology of CNS-Hormone Interactions" held in Chapel Hill, North Carolina (May 14-16, 1974).

Publications:

Kostrzewa, R. and Jacobowitz, D.: Acute effects of 6-hydroxydopa on central monoaminergic neurons. Eur. J. Pharmacol. 21: 70-80, 1973.

Jacobowitz, D., Kent, K.M., Fleisch, J.H. and Cooper, T.: Histofluorescent study of catecholamine-containing elements in cholinergic ganglia from the calf and dog lung. Proc. Soc. Exp. Biol. Med. 144: 464-466, 1973.

Jacobowitz, D. and Greene, L.A.: Histofluorescence study of chromaffin cells in dissociated cell cultures of chick embryo sympathetic ganglia. J. Neurobiol. 5: 65-83, 1974.

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Dubois, A., Kopin, I.J., Pettigrew, K.D. and Jacobowitz, D.M.: Chemical and histochemical studies of postoperative sympathetic activity in the digestive tract. Gastroenterology 66: 403-407, 1974.

Chase, T.N., Balch, T.S. and Jacobowitz, D.M.: Dopamine histofluorescence in postmortem specimens of human and rat striatum. Symposium on Catecholamines and Their Enzymes in Neuropathology of Schizophrenia (in press), 1974.

Raezer, D.M., Wein, A.J., Jacobowitz, D.M. and Corriere, J.N.: Autonomic innervation of canine urinary bladder. Cholinergic and adrenergic contributions and interaction of sympathetic and parasympathetic nervous systems in bladder function. Urology, 2: 211-221, 1973.

Patil, P.M. and Jacobowitz, D.M.: Significance of unequal accumulation of adrenergic drugs in the pigmented and the nonpigmented iris. Amer. J. Ophthal. (in press), 1974.

Wein, A.J., Jacobowitz, D.M., Corriere, J.N. and Murphy, J.J.: Chemical sympathectomy with 6-hydroxydopamine: A useful research tool. Surgical Forum, 24: 560-562, 1973.

Kobayashi, R.M., Palkovits, M., Kopin, I.J. and Jacobowitz, D.M.: Biochemical mapping of noradrenergic nerves arising from the rat locus coeruleus. Brain Res. (in press), 1974.

Dubois, A. and Jacobowitz, D.M.: Failure to demonstrate serotonergic neurons in the myenteric plexus of the rat. Z. Zellforsch. (in press), 1974.

Jacobowitz, D.M. and Palkovits, M.: Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the brain. I. Forebrain (Telencephalon, Diencephalon). J. Comp. Neurol. (in press), 1974.

Palkovits, M. and Jacobowitz, D.M.: Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the brain. II. Hindbrain (Mesencephalon, Rhombencephalon). J. Comp. Neurol. (in press), 1974.

Articles Published in Books:

Jacobowitz, D.: The peripheral autonomic system. In: Hubbard, J.I. (Ed.): The Peripheral Nervous System, 1974.

Jacobowitz, D.: Effects of 6-hydroxydopa. In: Frontiers in Catecholamine Research, ed. E. Usdin, S. Snyder, Pergamon Press, London, 1973.

Serial No. M-CS-ET-1
1. Laboratory of Clinical Science
2. Section on Experimental Therapeutics
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemistry, Pharmacology and Physiology of Monoamines and Other Central Neurotransmitters

Previous Serial Number: Same

Principal Investigator: Thomas N. Chase

Other Investigators: Monte S. Buchsbaum, Jacob A. Brody, Anne C. Carter, Joseph Fermaglich, George A. Glaubiger, Edna K. Gordon, David A. Jacobowitz, Irwin J. Kopin, Morris A. Lipton, Charles E. Morris, Lorenz K.Y. Ng (Guest Worker), E. Williams Pelton (Guest Worker), Jorge Perez-Cruet, Eugene Roberts, Ira Shoulson and Roger Weir (Guest Worker)

Cooperating Units: Sections on Medicine and Psychiatry and Units on Clinical Biochemistry and Histopharmacology, Laboratory of Clinical Science and Perceptual and Cognitive Studies Section, Adult Psychiatry Branch, NIMH; Epidemiology Branch, NINDS; Department of Neurology, Georgetown University School of Medicine; Departments of Neurology and Psychiatry, North Carolina School of Medicine; Department of Medicine, Downstate Medical Center, New York; City of Hope National Medical Center, Duarte, California

Man Years:

Total	5.0
Professional	3.0
Other	2.0

Project Description:

Objectives:

1. To study the transport, metabolism, storage, release and post-synaptic interactions of monoamines and other putative central neurotransmitters.

2. To examine the functional role of monoamines and other suspected neurohumoral agents in the central nervous system of normal individuals and in patients manifesting neuropsychiatric disease.

3. To study the mechanism of action and therapeutic efficacy of drugs which modify brain function.

Methods Employed:

1. Preclinical studies utilize a) slices or homogenates of brain, peripheral nerve and other tissues which are incubated with radioactively labeled amines and related compounds, then exposed to drugs or electrical stimulation· b) surgically prepared laboratory animals in which the concentration of labeled and endogenous compounds in brain, cerebrospinal fluid, serum and urine are determined under various experimental conditions following the administration of labeled amines and other substances.

2. Clinical studies are conducted in patients with neuropsychiatric disease and in normal volunteers. Under basal conditions and during the administration of drugs or physical procedures believed to influence central synaptic mechanisms, neurologic, psychiatric and psychologic parameters of brain function are correlated with the results of a) chemical assay of relevant compounds in various body fluids and tissues. b) radioisotope tracer procedures and c) electrical measures of spontaneous and evoked cortical potentials.

Major Findings: Preclinical investigations conducted with Dr. G.A. Glaubiger on central cholinergic mechanisms continue. Previous work has shown that lithium chloride reduces the accumulation of tritiated choline and acetylcholine in rat brain produced by the systemic administration of radiolabeled choline. In vitro studies now indicate that lithium ions competitively inhibit the uptake of choline into rat brain synaptosomes but have no effect on the activity of choline acetyltransferase, acetylcholinesterase, or on the electrically-induced release of labeled acetylcholine. Pharmacologic and radioisotopic methods for estimating the central turnover of γ-aminobutyric acid (GABA) have also been developed during the past year and applied to studies of various psychoactive drugs. Neither acute nor chronic treatment with L-dopa was found to alter GABA turnover.

An examination of changes in the ability of central neurons to accumulate and release monoamines during postnatal development has been extended by Dr. E.W. Pelton. Maturation rates for uptake mechanisms for norepinephrine, dopamine and serotonin show substantial regional differences. Moreover, the ability of rat brain to concentrate monoamines appears to attain adult characteristics long before maturation of mechanisms subserving stimulus induced release is complete. Dr. Pelton is also continuing to evaluate the possible role of endogenously synthesized 6-hydroxydopamine as a autotoxin in Parkinson's disease and related disorders characterized by a selective degeneration of dopaminergic neurons.

A single blind clinical trial of a new putative dopamine receptor stimulating agent, ET-495, has been completed in 16 patients with idiopathic parkinsonism. At maximum dose levels overall improvement in parkinsonian signs averaged 30%. Adverse effects resembled those associated with L-dopa. The antiparkinsonian efficacy of L-dopa together with a peripheral decarboxylase inhibitor, tested in 12 of these patients, was about twice that of ET-495.

As expected of a dopamine receptor agonist, ET-495 substantially reduced the central turnover of this monoamine as estimated by the probenecid loading test. The relatively modest antiparkinsonian efficacy of ET-495 may be due to the fact that it acts only as a partial dopamine receptor agonist. Experiments to test this hypothesis are now in progress. Preliminary results suggesting that ET-495 suppresses involuntary movements in some patients with tardive dyskinesias would appear to support this contention.

Last year we observed that the brittle "on-off" response to L-dopa in parkinsonian patients could be overcome by maintaining constant circulating dopa levels by means of a continuous intravenous infusion of the amino acid. A clinical trial of a commercially available "sustained" release L-dopa preparation (Astrodopa) nevertheless, failed to stabilize the response of any of the parkinsonian patients tested. Plasma assays subsequently revealed that fluctuations in circulating dopa concentrations were not significantly attenuated by this preparation. A contract to formulate an improved sustained release L-dopa preparation has now been let in cooperation with the NIH Pharmaceutical Development Service.

Recent clinical studies with Drs. G.A. Glaubiger and I. Shoulson suggest that noradrenergic mechanisms may also contribute to the "on-off" response to orally administered L-dopa. Procedures which activate the sympathetic nervous system such as sudden tilting to the vertical position or the cold-pressor test were found to dramatically reduce L-dopa's antiparkinsonian efficacy. Plasma norepinephrine levels at these times were significantly increased. Since intravenously infused norepinephrine had no effect on the antiparkinsonian response to L-dopa, norepinephrine release outside the central nervous system would not appear to mediate these changes.

In view of preclinical evidence suggesting that the activity of norepinephrine containing neural pathways influences mammalian motor behavior and that the centrally mediated effects of L-dopa may in part be attributable to changes in noradrenergic function, a single blind trial of fusaric acid was conducted in 15 patients with idiopathic parkinsonism. At doses which appeared to modify the central metabolism of norepinephrine, this dopamine-β-hydroxylase inhibitor failed to alter parkinsonian severity in otherwise untreated patients. Fusaric acid also had no consistent effect on the ability of L-dopa to ameliorate parkinsonian signs or to induce dyskinesias. These results do not support the contention that noradrenergic mechanisms play a critical role in the pathophysiology of Parkinson syndrome or in the extrapyramidal effects of L-dopa in parkinsonian patients.

As a further test of the role of adrenergic systems in the regulation of extrapyramidal function in man the ability of clonidine, an α-adrenergic stimulating agent, to modify the response to ET-495 is being studied in patients with various extrapyramidal disorders. Although clonidine has been reported to potentiate the motor effects of L-dopa in rodents, preliminary results in man suggest quite the opposite response. Additional studies of central adrenergic mechanisms in L-dopa treated parkinsonian patients using

norepinephrine receptor blocking agents such as phenoxybenzamine or propranolol have also recently been initiated.

A study of the effect of diet on brain serotonin metabolism, completed in collaboration with Drs. J. Perez-Cruet and D.L. Murphy, indicates that there is an inverse relationship between total plasma levels of the serotonin precursor, tryptophan, and the spinal fluid content of this amino acid as well as of the serotonin metabolite, 5-hydroxyindoleacetic acid. Our results suggest that serotonin formation in brain may depend on such factors as the binding of tryptophan to plasma proteins, the concentration of plasma triglycerides, and levels of other neutral amino acids which compete with tryptophan for transport into the central nervous system.

Considerable preclinical evidence would appear to link the function of serotonin containing neural systems to the regulation of certain behavioral activities. An examination of the behavioral changes which occur during the administration of drugs which selectively influence serotonin mediated function provide one approach to the study of these relationships in man. Together with Dr. I. Shoulson a study of biochemical and behavioral changes attending a therapeutic trial of fenfluramine, a drug known to facilitate serotonin mediated function in the experimental animal, is now in progress. In 8 patients fenfluramine was found to significantly reduce caloric intake and body weight, but to increase rating scores for depression and somnolence. This drug had no effect on involuntary movements in patients with Huntington's disease. Treatment with fenfluramine lead to a substantial decline in central serotonin turnover, as estimated by the oral probenecid technique, in all patients tested: there was no consistent change in dopamine metabolism. These results are consistent with observations in the experimental animal and suggest that fenfluramine may act to stimulate postsynaptic serotonin receptors or to block the reuptake of serotonin released into the synaptic cleft.

A double blind crossover trial has been conducted in 6 patients with Huntington's disease to assess the therapeutic efficacy of lithium. No improvement in motor or cognitive function occurred. There was, however, an apparent clinical worsening during the first few days of treatment and during a similar period immediately following lithium withdrawal. This functional deterioration was accompanied by a transient decline in serum calcium levels.

Recent reports of diminished GABA and glutamic acid decarboxylase levels in the brains of patients with Huntington's disease may provide a lead to the pathogenesis of the motor and behavioral signs of this hereditary disorder. Since GABA may serve as a neurotransmitter in the striatal and cortical interneurons which degenerate in Huntington's disease, drugs which facilitate GABA mediated transmission might be expected to ameliorate the symptoms of this disorder. Preclinical studies suggest that imidazole-4-acetic acid acts as a GABA agonist in the mammalian central nervous system. A brief, therapeutic trial of this substance failed, however, to produce any alteration in motor or cognitive function. It remains to be determined whether this result is due to pharmacodynamic factors or to an inherent inability of GABA replacement

therapy to modify the signs of Huntington's disease. The next step in these studies, conducted with Drs. I. Shoulson and E. Roberts, will be to give the isopropyl ester of imidazole acetic acid in an attempt to increase the central nervous system penetration of the orally administered drug. Preliminary results from a related drug evaluation suggest that the parachlorophenyl derivative of GABA (BA-34,647) also may not provide symptomatic relief to patients with Huntington's disease.

Both preclinical and clinical studies of the role of monoamine containing neural systems in the pathogenesis of epilepsy have begun in collaboration with Dr. I. Shoulson. A colony of gerbils with a hereditary, stimulus-sensitive seizure disorder has been established. Clinically, therapeutic trials of a putative dopamine receptor agonist (ET-495), a serotonin receptor agonist (fenfluramine) and of the amino acid, taurine, have been initiated. The rationale for these investigations derives from observations in the experimental animal suggesting that drugs which increase monoamines in brain elevate seizure thresholds, while pharmacologic agents which depress central monoaminergic function increase the likelihood of experimentally induced seizures.

Recent preclinical observations indicate that certain hypothalamic releasing factors may influence mammalian motor behavior. For example, both thyrotropin releasing hormone and melanocyte stimulating hormone release inhibiting factor have been reported to potentiate L-dopa induced hypermotility in rodents. The intravenous infusion of thyrotropin releasing hormone had no apparent effect on extrapyramidal function in 5 otherwise untreated parkinsonian patients nor did it significantly modify the clinical response to L-dopa in 6 other individuals. Melanocyte stimulating hormone release inhibiting factor exerted a mild amelioratory effect in 3 of 6 untreated patients, while in 5 L-dopa treated individuals antiparkinsonian efficacy was potentiated in 2 and dyskinesias increased in 3. In neither group, however, did these changes attain statistical significance. In view of our results with melanocyte stimulating hormone release inhibiting factor, clinical trials of this tripeptide, given orally in high doses, have now been initiated at the Clinical Center and on Guam.

The ability of monoaminergic systems to influence the release of human pituitary hormones has also been examined during the past year with Drs. I. Shoulson and A.C. Carter. Like L-dopa, ET-495, a putative dopamine receptor agonist, was found to depress circulating prolactin levels but increase plasma concentrations of growth hormone. On the other hand, fenfluramine, a suspected serotonin receptor agonist, suppressed the release of growth hormone but induced a significant rise in prolactin. Neither drug significantly altered TSH, FSH, or LH levels. These preliminary observations suggest that dopaminergic and serotonergic systems may act reciprocally on the release of certain pituitary hormones.

In collaboration with Dr. M.S. Buchsbaum, a controlled study of the analgesic efficacy of acupuncture has been conducted in a series of normal

volunteers. A computerized system allows the amplitude of a random series of electrical stimuli, delivered to one forearm, to be rapidly correlated with patient reports of stimulus intensity and associated alterations in the amplitude of cortical evoked potentials. Thus far no difference has been discerned between the ability of a "placebo" and "active" needle placements to modify either the subjective or objective response to stimulation.

Significance to Biomedical Research and the Program of the Institute: The Section seeks to develop by rational means improved pharmacologic treatments for neuropsychiatric disease. Experimental efforts continue to largely focus on neurohumoral mechanisms, since substances participating in this process appear to act as sensitive indicators of central nervous system function and constitute the site of action of many drugs which affect psychomotor behavior. Alterations in central monoamines found in relation to several extrapyramidal and behavioral disorders have lead to the discovery of drugs which reverse both the biochemical and clinical defects. This approach to the development of new pharmacologic therapies may be applicable to other disorders of brain function.

Proposed Course of Project: Studies of monoamines in relation to naturally occurring and drug-induced neuropsychiatric disease will continue. Attempts to improve our ability to evaluate central monoaminergic mechanisms in man by means of an intravenous probenecid test alone or combined with non-radioactive isotopic tracer infusions will be undertaken. The role of monoaminergic and other neurohumoral systems in mediating pain perception will also be examined. Preclinical studies and clinical trials of new dopamine and GABA receptor agonists will be pursued actively.

Honors and Awards: During the past year Dr. Chase presented invitational lectures or seminars at the Tenth International Congress of Neurology, Barcelona, Spain, September 9-14, 1973; Michigan Chapter, Committee to Combat Huntington's Disease, Ann Arbor, October 28, 1973; American College of Neuropharmacology, Palm Springs, California, December 4-7, 1973; Huntington's Chorea Workshop, Los Angeles, California, January 11-13, 1974; Department of Neurology, University of Maryland School of Medicine, Baltimore, February 15, 1974; New York City Chapter, Committee to Combat Huntington's Disease, March 29, 1974; Maryland Chapter, Committee to Combat Huntington's Disease, April 1, 1974; American Academy of Neurology, Special Courses on Cerebrospinal Fluid, San Francisco, April 23, 1974, and to the Departments of Neurology and Neurosurgery, George Washington School of Medicine, May 25, 1974. Dr. Chase also conducted a workshop on Huntington's chorea and related genetically determined disorders at NIH for post doctoral fellows from universities across the country on October 12-14, 1973 and he served as principle organizer, program committee co-chairman and proceedings co-editor for an international congress on the "Dopamine Receptor", held in Paris, May 9-11, 1974. In addition, Dr. Chase was an invited speaker at conferences on "L-dopa and Cognitive Function", West Point, Pennsylvania, August 17, 1973, "Tardive Dyskinesias", Spring Grove State Hospital, October 22-23, 1973 and on "Fenfluramine and its Derivatives", Marbella, Spain, March 9-16, 1974.

Publications:

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Serial No. M-CS-PS(C)-23
1. Laboratory of Clinical Science
2. Section on Clinical Neuropharmacology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical neuropharmacology and psychobiology of human behavioral disorders, especially mania and depression.

Previous Serial Numbers: M-CS-PS(C)-18,20

Principal Investigator: Dennis L. Murphy

Other Investigators: Eugene D. Brand (Visiting Fellow), Jonathan L. Costa, Daniel P. van Kammen (Visiting Fellow), Donald E. Redmond and Joseph Tarantolo

Collaborating Scientists: Robert H. Belmaker, Monte S. Buchsbaum, William E. Bunney, Jr., Edward F. Donnnelly, Frederick K. Goodwin, George M. Henry, Halbert B. Miller, Herbert Weingartner, Richard J. Wyatt.

Psychiatric Consultants: Marvin L. Adland, John Boriello, Marion B. Richmond, Leslie Schaffer

Cooperating Units: Nursing personnel on 3-East; Occupational Therapy (Eleanor A. Stapin); Art Therapy (Sadie Fishman) and Social Work personnel (Carol F. Hoover, Walter Sceery) on Ward 3-East

Man Years:

Total	9.7
Professional	5.2
Other	4.5

Project Description:

Objectives: Individuals with mania, depression and related disorders are studied in an attempt to understand the mechanisms of therapeutic drug effects in these disorders.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, and from direct behavioral observation. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed on this unit.

2. Biological Assessment: Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of enzymes, levels of biogenic amines and their metabolites, other chemical variables, and drug levels. Electrophysiologic measurement of sleep and of cortical evoked potentials are accomplished in collaborative studies with the Laboratory of Clinical Psychobiology and the Adult Psychiatry Branch.

Major Findings:

Behavioral and biological effects of L-tryptophan, lithium carbonate, d- and l-amphetamine, physostigmine and other drugs in man. L-tryptophan was reported last year to be ineffective as a antidepressant in unipolar patients; however continued study of this agent in collaboration with Drs. Bunney and Goodwin revealed that bipolar patients manifested improvement in behavior, mood and sleep when treated with L-tryptophan during both depressed and manic phases. This therapeutic effect may reflect a modulating interaction of brain serotonin with brain catecholamine systems. L-tryptophan administered in 8-12 g/day doses also had a moderate enhancing effect on memory in depressed patients. This effect was not as striking as that observed with L-dopa. Further studies of the effect of L-dopa on memory, accomplished with Dr. Henry and Dr. Buchsbaum, indicate that the improvement observed with L-dopa depends on long-term, high dosage treatment, as acute intravenous injections of L-dopa in MK-486-pretreated patients produced no memory or behavioral effects, although definite cortical evoked response changes were elicited.

Continuing studies of the effects of lithium carbonate in man revealed that lithium administration is accompanied by a temporary diminution in the release of thyroxin from the thyroid gland and a dampening of the normal circadian variation in thyroxin release. These hormonal studies of lithium represent the third year of collaborative studies with the NIAMD, Clinical Endocrinology Branch (Dr. J. Robbins and Dr. H. S. Carlson).

In ongoing studies, differential behavioral effects of d- vs l-amphetamine were obtained in a study attempting to determine whether amphetamine responses are predictive of tricyclic drug responses in depressed patients, in a study carried out with Dr. van Kammen. Although amphetamines are not effective antidepressants during long-term administration, most depressed patients have marked euphoriant, activating and antidepressant responses to single dose d-amphetamine administration. In a pilot study carried out with Dr. Redmond, the cholinergic agent physostigmine given together with a peripherally-acting anticholinergic agent was observed to markedly diminish some features of manic behavior. This evidence lends further support to the necessity for considering the interaction of other neurotransmitter systems in brain (i.e., the cholinergic and serotonergic systems) as possibly involved in the CNS regulation of affect and affect-related behavior.

Affect- and drug-state dependent learning. As part of a continuing study of the interactions between psychoactive drugs, affect changes and learning and memory led by Dr. Weingartner, ten patients with affective disorders have been studied longitudinally to determine whether a diminished transfer of information from one affective state to another and from a drug to a non-drug state might occur. The initial results indicate a strong inverse relationship between the retreival of words or the ability to recognize pictures and the magnitude of rated change in affect. These results may help explain some of the cognitive effects of drugs which have antidepressant and antimanic actions. In addition, they suggest the possibility that the state-dependent learning effects of other psychoactive drugs might be mediated through affective changes.

Drug response differences in bipolar and unipolar depressed patients as related to personality characteristics. These studies, which have been accomplished in collaboration with Dr. E. Donnelly involved comparisons of unipolar and bipolar depressed patients on MMPI subscales, the Rorschach procedure, and other psychological tests. They revealed evidence of different personality characteristics in these two depressed patient subgroups which are consistent with both clinical impressions and with some observed drug responses. For example, unipolar depressed patients are generally more anxious, more somatically concerned and have less marked responses to stimulant drugs, while bipolar patients are, on the surface, less "neurotic," less concerned with psychological phenomena, more concerned about what others think of them, and respond to L-dopa and amphetamine with markedly increased activity and other behavioral phenomena.

Platelet monoamine oxidase and studies of the cellular effects of psychoactive drugs in man utilizing the platelet model.

Studies of human monoamine oxidase, which had originally revealed marked changes in individuals treated with MAO-inhibiting antidepressant drugs and some differences in psychiatric patients, including schizophrenic and bipolar depressed individuals, have continued in two major areas. Large numbers of normal individuals have been investigated to assess such factors as age, sex, and hormonal factors, including the menstrual cycle, which have been verified in these studies with Drs. Belmaker and Wyatt as being capable of affecting platelet MAO activity. Additionally, *in vitro* studies with multiple subtrates and inhibitors as well as other approaches have identified the human platelet MAO as a Type B MAO which is distinct from human plasma MAO and which appears very similar, if not identical to the B component of MAO found in other tissues, including brain.

New findings concerning the effects of drugs in man at the cellular level include evidence that lithium carbonate treatment is associated with reduced platelet receptor responses to prostaglandin E_1 and norepinephrine. Cellular cyclic AMP changes produced by these hormones are also altered *in vitro* in a dose-dependent fashion, although somewhat greater lithium concentrations are required. Lithium appears to block other hormones which act via cyclic AMP, and these effects may provide alternative or additional explanations (besides lithium's biogenic amine interactions) for some of the psychoactive effects and side effects of lithium treatment. Other studies of the platelet model quantified serotonin storage packet size in human platelets, utilizing an electron microscopic approach carried out by Drs. Costa and Reese, and demonstrated for the first time the occurrence of octopamine in human cells. Although small amounts of this amine, which may function as a "false neurotransmitter," had been found in human urine, and preliminary studies indicated only trace amounts in normal individuals, much larger amounts were positively identified in platelets from depressed patients receiving MAO-inhibiting drugs in a study carried out with Drs. Cahan and Molinoff, using a sensitive assay for octopamine developed by Drs. Molinoff and Axelrod.

Significance to Biomedical Research and the Program of the Institute:

These studies bear directly on our understanding of the mode of action of some psychoactive drugs used in the treatment of depression and mania. They suggest that several central neuromediator systems (catecholaminergic, serotonergic and cholinergic) as well as thyroid hormone and sex steroids affect behavior and may be involved in the effects of drugs such as lithium. They continue to emphasize the importance of differentiating bipolar and unipolar subgroups of depressed patients in studying drug responses. They also illustrate the

application of the platelet model to the definition of biochemical effects of drugs in individual patients.

Proposed Course of the Project:

Continuation of these integrated studies of manic and depressed patients will be focused on evaluating individual behavioral differences in response to drugs in relation to preexisting personality and biological (e.g., blood cell enzymes) characteristics and to differences in the metabolism and final biochemical effects of drugs.

Honors and Awards:

Dr. Murphy was invited to present lectures on the psychobiology and psychopharmacology of mania and depression at Rutgers University School of Medicine, at Georgetown University, at Howard University, at the University of Florida and at the Shepherd-Pratt Hospital. He presented a seminar on clinical psychopharmacology at Johns Hopkins Hospital and also conducted a Grand Rounds presentation on the behavioral effects of L-dopa organized by the Johns Hopkins Hospital Department of Neurology. He was invited to present papers on various aspects of studies of monoamine oxidase and platelet pharmacology at the annual meetings of the American Association for the Advancement of Science and the American College of Neuropsychopharmacology as well as at Yale University. He discussed the relevance of aminal psychopathological models to the human at an international symposium sponsored by the Kittay Foundation.

Dr. Redmond was invited to give a lecture on studies of primate social behavior after pharmacologic alterations of brain monoamines at the University of North Carolina School of Medicine.

Dr. Costa was invited to present seminars on amine secretion by platelets at the Medical College of Pennsylvania and the Downstate Medical Center, SUNY. He presented lectures on exocytosis and endocytosis in the perfused adrenal medulla at the Hershey Medical Center, State University of Pennsylvania, and at the George Washington University Medical School.

Dr. Henry presented a paper on the effects of intravenous L-dopa on learning, cortical evoked responses and behavior at the annual meeting of the American Psychosomatic Society.

Dr. Weingartner presented papers on learning and human state-dependent learning in relation to drug effects and affect changes at symposia at the American Psychological Association meeting and the Winter Brain Research Conference.

Publications:

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Serial No. M-CS-Ps(C)-19
1. Laboratory of Clinical Science
2. Section on Psychiatry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Brain Amine Function in Mental Illness: Studies of Cerebrospinal Fluid and Urinary Amine Metabolites

Previous Serial Number: Same

Principal Investigator: Frederick K. Goodwin

Other Investigators: Robert Sack, Helmut Beckmann

Collaborating Scientists: Robert Post, Michael Ebert, Dennis Murphy

Cooperating Units: 4-West, 3-East, and 3-West Clinical Research Units, Unit on Clinical Biochemistry, Laboratory of Clinical Science

Man Years:

Total	3.0
Professional	1.7
Other	1.3

Project Description:

Objectives: The objectives of this project is to continue development of methods for the estimation of neurochemical alterations in patients by assessment of amine metabolites in the cerebrospinal fluid and the urine. The objectives include exploration of both the potential and limitations of body fluid metabolite studies and some of the non-specific factors which effect them.

Methods Employed:

a. Cerebrospinal Fluid (CSF) Studies: Sampling of the CSF is accomplished by lumbar puncture. The position of the patient, time of day, and antecedent activity have all been examined for a possible effect of the metabolite levels under study. Headache, a major side effect of lumbar puncture, has been considerably reduced by alteration of the conditions found to contribute to headache.

As an adjunct to the CSF studies, the drug probenecid is employed. This drug inhibits the active transport of acid metabolites of brain amines, 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA), the major breakdown products of serotonin and dopamine respectively. CSF levels of these acid metabolites are closely related to brain levels and their rate of

accumulation during probenecid administration can be used as an indirect index of amine turnover. The neurochemical assessment of the CSF amine metabolites involves spectrophotofluorimetry for 5HIAA and HVA, gas-liquid chromatography for 3-methoxy-4-hydroxyphenylglycol (MHPG -- the major metabolite of norepinephrine in brain) and (in collaboration with Mrs. Gordon of the Unit on Clinical Biochemistry) both MHPG and HVA are additionally being analyzed by mass spectroscopy; these results are correlated with either the fluorometric or gas chromatographic method.

b. Urinary Amine Metabolite Studies: Urine is collected daily on each patient in 24-hour pools which are kept refrigerated with preservative until aliquotted into frozen samples within three days. Conditions which can produce alterations in urinary amines and their metabolites are monitored or controlled to the best of our ability; these include diet, exercise, and stress. Urine is analyzed for a variety of amines and their metabolites, principally MHPG, the metabolite in urine which probably best reflects brain norepinephrine metabolism.

Major Findings

(1) CSF Studies:

a. Continued data on baseline levels of amine metabolites in affective illness and schizophrenia has been collected in collaboration with Dr. Robert Post of the Adult Psychiatry Branch. Diagnostic subtypes, severity of symptom, type of symptom, subsequent drug response, and other clinical features have all been examined for possible correlations with baseline levels of amine metabolites in affective illness. The major finding continues to be that, as a group, the depressed patients have a substantially lower MHPG in the CSF when compared to controls, manic patients, or to a group of acute schizophrenics studied in collaboration with the Adult Psychiatry Branch.

b. Probenecid induced accumulation of amine metabolites indicate that 5HIAA accumulation is lower in both the depressed and manic group when compared to normal controls. Frequency distribution analysis of the individual data indicates that for both the MHPG and the probenecid 5HIAA, the lower mean results from a small subgroup with very low values. Overall, there is no relationship between MHPG and 5HIAA accumulation, although a small subgroup is very low in both; although it has not yet been possible to specifically identify any clinical features which might correspond to this subgroup, it is our clinical impression that they represent some of the patients with the most severe symptoms, particularly those with involutional features.

c. The low MHPG found in many of our patients during depression continues to be low in the immediate post-recovery period just prior to discharge for the hospital. This persistence of low MHPG appears to be independent of mode of treatment (drugs, ECT, or recovery without physical treatment).

d. Prebenecid induced accumulation of 5HIAA is reduced following a variety of antidepressant treatments, including tricyclics, lithium, or ECT. This reduction in serotonin "turnover" following treatment of depression is paradoxical since these patients have a lower mean serotonin "turnover" to start with. This finding may provide clinical evidence for the suggestion which has been made on the basis of animal studies that behavioral response to psychotrophic drug of this type may be more related to the neurochemical adaptation to the initial effect of a drug, adaptive responses involving feedback regulatory mechanisms.

2. Urinary Amine Metabolite Studies:

a. The excretion of urinary MHPG has been found to increase substantially when patients participate in a moderate exercise protocol (the mean increase in urinary MHPG excretion during exercise may result from the exercise itself, from stress, or from a combination or both; these findings suggest caution in the interpretation of MHPG changes in manic-depressive and recovered patients in which large differences in physical activity occur.

b. MHPG responses to stress in depressed versus normal individuals: in collaboration with Dr. Buchsbaum (Laboratory of Psychology) urinary MHPG excretion has been examined following pain-induced stress in 20 normal volunteers and in a smaller group of depressed patients. Preliminary findings suggest that among the normal volunteers there is a slight increase in urinary MHPG when the stress day is compared to a control day; the increase in MHPG correlates highly ($r = 0.89$) with pre-stress depression scores on the MMPI. When compared to the slight MHPG response to stress in normals, depressed patients show an exaggerated increase in urinary MHPG following pain-induced stress.

c. The use of differential MHPG excretion in the prediction of response to different antidepressants: we have found that depressed patients who subsequently show an antidepressant response to amitriptyline (Elavil) have high pretreatment levels of MHPG when compared to those who do not show a response; the converse was found for imipramine (Tofranil) with responders having higher pretreatment levels than nonresponders. Although the number of patients in these studies are relatively small (a total of 15 patients) they were selected because of the unequivocal nature of the clinical response; the fact that there is no overlap between individual pretreatment MHPG values in the responder or nonresponder groups for both drugs suggest the potential clinical usefulness of urinary MHPG as a prediction of tricyclic drug response.

d. The effect of tricyclic antidepressant treatment on urinary MHPG in depressed patients: the overall effect of treatment with either imipramine or amitriptyline was a decrease in urinary MHPG when the third week of treatment was compared with the pretreatment week. This decrease was independent of clinical response. Additional studies are underway on the effects of lithium and amphetamine administration on urinary MHPG in depressed patients.

Proposed Course of the Project: CSF -- Urine Studies. A major focus will be in continued efforts to improve methods for the direct study of brain amine function in man. More specifically, the relationship between plasma and CSF probenecid levels and amine metabolite accumulation will be further explored. In collaboration with the Unit on Clinical Biochemistry, we will continue to study the correlations between various assay techniques for amine metabolites.

In the clinical areas, emphasis will be given to long term studies, particularly to see if the low CSF metabolite levels found in the immediate post-recovery period will persist indefinitely (and therefore possibly reflect an underlying predisposing factor), or will gradually return to normal.

Both the CSF and the urinary amine metabolite values will be correlated with clinical subtypes and response to different treatment modalities, in a continued effort to explore the potentials and limitations of a "biochemical subtyping" approach to depressed patients and their pharmacological treatment.

Honors and Awards:

Dr. Goodwin: See Project Report No. M-CS-Ps(C)-22.

Dr. Sack was an invited speaker at the University of California at La Jolla where he presented a paper on "The Catecholamine Hypothesis Revisited." In addition he presented invited lectures at the University of California at Davis, and at the University of California at Irvine. He was also a guest lecturer at University of Washington in Seattle, and the University of Colorado in Denver and the Medical College of Virginia at Richmond. At the Annual meeting of the American College of Neuropsychopharmacology (ACNP), he participated in a panel and presented a paper entitled "The Catecholamine Hypothesis; a View from the Clinical Shores." He was co-author on a paper presented at the International Catecholamine Symposium in Strasbourg, France. In addition he presented a paper at the American Psychiatric Association on "The Effects of a DBH Inhibitor in Mania" and was a co-author on one additional paper at the meeting.

Publications:

Post, R.M., Kotin, J., Goodwin, F.K. and Gordon, E.K.: Psychomotor activity and cerebrospinal fluid amine metabolites in affective illness. Amer. J. Psychiat. 130:67-72, 1973.

Goodwin, F.K. and Ebert, M.: Lithium in Mania. In Lithium: Its Role in Psychiatric Research and Treatment. Gershon, S. (Ed), Plenum Press, New York, 1973.

Post, R.M. and Goodwin, F.K.: Effect of amitriptyline and imipramine on amine metabolites in the cerebrospinal fluid of depressed patients. Arch. Gen. Psychiat., 30:234-239, 1974.

Goodwin, F.K. and Post, R.M.: The effect of antidepressant treatment on central serotonin turnover in depressed patients. In 5-Hydroxy-tryptamine in Brain, Costa, E. and Gessa, G.L. (Eds.), Raven Press, 1973.

Post, R.M. and Goodwin, F.K.: Cerebrospinal fluid amine metabolites in affective illness. Proceedings of the 2nd Congress of the International College of Psychosomatic Medicine, S. Karger AG, Basel, Switzerland, 1973.

Serial No. M-CS-Ps(C)-22
1. Laboratory of Clinical Science
2. Section on Psychiatry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical and psychopharmacological studies of manic-depressive and schizo-affective disorders

Previous Serial Number: Same

Principal Investigator: Frederick K. Goodwin

Other Investigators: Robert Sack, Laurence Greenwood, Thomas Wehr, Helmut Beckmann (Visiting Associate), Jacques St.-Laurent (Guest Worker)

Collaborating Scientists: Robert Post, Dennis Murphy, Michael Ebert, Monte Buchsbaum, Robert Colburn, William T. Carpenter

Psychiatric Consultants: Arnold Meyersburg, Marion Richmond, Paul Chodoff, Marvin Adland, Alina Efron

Cooperating Units: Social Work Personnel on Ward 4-West (Yolande Davenport); Nursing Personnel and Occupational Therapist (Judy Schreiber) on 4-West

Man Years:

Total	5.8
Professional	3.5
Other	2.3

Project Description:

Objectives: The objectives of these studies is a more comprehensive understanding of manic-depressive and schizo-affective disorders, particularly in relation to specificity of diagnosis, existence of biologically identifiable sub-groups which may predict drug response, the nature of pre-disposing factors, and long-term outcome.

Methods Employed:

Behavioral Evaluation: After referral by physicians, patients are interviewed by an intake evaluation team of two physicians, a social worker and a psychiatric nurse; selection for admission is based on research criteria, i.e., primary affective (or schizo-affective) disorder severe enough to require hospitalization. Depressed patients generally show psychomotor retardation and/or agitation, sleep disturbance, loss of appetite with weight loss,

excessive tiredness, loss of interest, and thought patterns dominated by feelings of helplessness, hopelessness, and guilt. There is frequently a past history of depressive and/or manic episodes, and often a family history of mood disorders. Manic patients typically evidence increase psychomotor activity with an increased initiation and rate of speech, with a labile mood predominated by either euphoria or irritability, and a cognitive state characterized by expansiveness, grandiosity, and over-confidence; more severely manic patients may show severe dysphoria, with disorganized psychotic features which may be difficult to distinguish from schizophrenia.

Behavioral data is collected on a longitudinal basis throughout the course of hospitalization utilizing twice daily global ratings by a trained nursing research team; these ratings focus on depression, anxiety, psychosis, anger, and anxiety. Three times a week a special 34-item affective illness scale is scored for each patient by each member of the nursing staff; this multi-item scale is capable of generating clusters of behavior which are analyzed by computer. More intensive clinical data is obtained from individual psychotherapy sessions held on a 2-3 times a week basis. Information regarding the family is obtained through ongoing contact between the social worker and the patient's relatives. Sleep is recorded at half-hour intervals through the night on all patients.

Pharmacological Methodology: All drug trials are conducted under double-blind conditions with alternating active and placebo periods in a non-random design. The utilization of placebo substitution following active drug increases one's level of confidence in the efficacy of a compound in an individual patient. This strategy is important in the evaluation of drug effects because of the frequency of spontaneous remissions and exacerbations in manic-depressive illness.

Major Findings: The major findings from this project during the last year can be divided into six separate areas:

1. Studies of the overlapping clinical phenomenology of affective, schizo-affective and schizophrenic disorders (done in collaboration with the psychiatric assessment section of the Adult Psychiatry Branch) have revealed a group of sub-items of the World Health Organization Psychopathology Rating Scale which reliably differentiate manic-depressive patients from schizo-affective or schizophrenic patients, and which correlate with outcome and drug response. This work has led to the development of a scale for the differential diagnosis of excited states occurring in manic-depressive, schizo-affective and schizophrenic illness.

2. Follow-up Studies of Manic-Depressive Patients and Their Spouses: This group of studies, conducted primarily with outpatients, have focused on outcome and prognosis. Among a group of 47 patients who had been manic during hospitalization, one-third were found to have remained functionally impaired with moderate to severe affective symptoms interfering with their work, social and family life. Clinical phenomenology of the acute manic episode in this poor outcome group was not different from that in the good outcome group.

The interaction of ongoing psychotherapy and the prophylactic efficacy of lithium is also under investigation. Preliminary data suggests that those manic-depressive patients who are involved in ongoing couples therapy have a better prophylactic response to lithium in terms of frequency and severity of affective episodes when compared to patients maintained on lithium without ongoing conjoint psychotherapy.

Additional aspects of the follow-up studies deal with the correlations between age of onset and outcome criteria, and specific interpersonal aspects of the marriages of manic patients.

3. Evaluation of New Drugs in the Treatment of Mania:

a. Fusaric Acid: This potent and specific inhibitor of the enzyme dopamine-beta-hydroxylase has been evaluated for its potential antimanic effects. The rationale for this approach is based on the indirect evidence suggesting that excess brain norepinephrine may be involved in the pathophysiology of mania; thus, a decrease in brain norepinephrine secondary to DBH inhibition might reduce or alleviate manic symptoms. However, at doses sufficient to inhibit plasma DBH by 95%, and to decrease the norepinephrine metabolites in the cerebrospinal fluid, no therapeutic efficacy could be demonstrated in nine manic patients; however, among those patients whose mania was characterized by psychotic features, there was a consistent intensification of psychosis while on the DBH inhibitor. Fusaric acid provides a pharmacological tool for producing a reversible DBH deficiency in living patients, allowing a more reasonable evaluation of the hypotheses relating DBH deficiency to psychopathology, particularly that of schizophrenia.

b. Pimozide: A trial of this drug has been conducted in a small number of manic patients, with a majority showing a rapid and relatively specific antimanic response. Although preliminary, these observations are of considerable theoretical importance since animal data suggests that pimozide acts almost exclusively through blockade of dopamine rather than norepinephrine receptors; taken together with a number of other studies from this laboratory, the preliminary data on pimozide would suggest that central dopamine rather than norepinephrine may be more prominently involved in the pathophysiology of mania.

4. Evaluation of Antidepressant Treatments:

a. Potentiation of Tricyclic Antidepressant Response with T3 (Cytomel): Extending the work of Prange, who demonstrated the acceleration of antidepressant response to tricyclic drugs with the addition of small doses of T3, we have explored the effects of T3 addition in patients who have already been established as non-responders to tricyclic antidepressants by rigorous dose and time criteria. To date six out of seven tricyclic non-responders were converted to responders within three to seven days by the addition of 25 micrograms of T3.

b. Evaluation of Thyrotropin Releasing Hormone (TRH) as an Antidepressant: Nine patients were given intravenous infusions of six to 1200 micrograms of TRH in a cross-over design with placebo; no clear antidepressant effects of TRH was demonstrated; however, some subtle and transient effects were noted by a blind observer. The significance of these effects are under further evaluation.

c. Studies on the Combined Use of Lithium and Tricyclic Antidepressants in Bipolar Depressed Patients: A group of patients who have shown only a partial or minimal antidepressant response to lithium alone or to tricyclic antidepressants alone were given these drugs in combination. In the majority of these patients a satisfactory antidepressant response to the drug combination was demonstrated.

d. Attempts at subgrouping depressed patients according to response to specific drugs has continued; further evidence has been accumulated suggesting that the antidepressant response to lithium is significantly more likely to occur in bipolar than in unipolar patients, whereas the unipolar/bipolar dichotomy in relation to tricyclic response is not as clear. Another approach involves biological subtyping of patients as a predictor of drug response; these studies, utilizing urinary and spinal fluid amine metabolites are described under project number M-CS-Ps(C)-19.

5. Psychodynamic Studies: Employing traditional psychodynamic concepts (ego-function, defense mechanisms, etc.) 20 consecutive patients have been examined throughout the course of hospitalization, particularly during periods of change from depression to mania or into euthymia. A strikingly similar constellation of unconscious conflicts was noted centering around chronic and massive impoverishment of relationships, the denial of all feeling about this and the breakdown of this defense prior to depressive or manic episodes. Antidepressant or antimanic medications appear to facilitate the reestablishment of defensive patterns, often on a somewhat more resilient basis.

6. In collaboration with the Section on Clinical Neuropharmacology (Dr. Murphy), continued developments of the multi-item computer scored affective disorder rating scale has focused on an evaluation of reliability and on correlations between individual items and/or clusters of items with the global scores for mania, depression, and psychosis.

Proposed Course of the Project: Efforts will continue towards perfecting more reliable diagnostic and rating instruments for affective illness, schizoaffective illness and schizophrenia.

Additional emphasis will be given to long-term follow-up studies of affective illness, using expanded outpatient facilities. In conjunction with this, biological and psychosocial studies of the first degree relatives of index patients, initiated this year, will be expanded.

New drugs for the acute treatment of depression and mania will be studied with particular emphasis on drug combinations and on the potentiation of therapeutic responses with various endocrine substances.

Evaluation of some of the psychobiological concomitants of chronic schizophrenia have been initiated, and will be accelerated.

Honors and Awards:

Dr. Goodwin was elected to Fellowship in the American College of Psychopharmacology, to membership in the Collegium Internationale Neuro-Psychopharmacologicum (CINP), and was appointed to the Task Force on Research Training, Council of Medical Education and Career Development, American Psychiatric Association. Dr. Goodwin presented a paper at the Psychiatric Research Society on "Cerebrospinal Fluid Amine Metabolites in Affective Disorder"; he served as the principal discussant and main summarizer for the International Phenothiazine Meeting in Bethesda.

At the Annual Meeting of the American College of Neuropsychopharmacology (ACNP), Dr. Goodwin was Chairman of the study group on "Amines and Affective Disorder: The Catecholamine Hypothesis Revisited", and presented or co-ordinated three papers in that study group. In addition, at the ACNP meeting he was an invited participant in the study group on Brain Dopamine, presenting a paper on "Brain Dopamine and Central Activation in Affective Illness". At the Annual Meeting of the American Association for the Advancement of Science, Dr. Goodwin was an invited participant in two symposia: in one on "Neuro-biological Mechanisms of Adaptation and Behavior" he presented a paper entitled "Clinical Evidence for Neurotransmitter Adaptation in Response to Anti-depressant Therapy", and in the second symposium on "Implications of Some Recent Biological Studies of Depressive Illness" he presented a paper on "The Role of Biogenic Amines in Depression, Direct Studies in Man"; in addition, Dr. Goodwin served as Chairman of one of the sessions in these symposia.

Dr. Goodwin was invited by the Royal College of Physicians of Canada to participate in a joint symposium with the Society for Clinical Pharmacology and Therapeutics in Toronto. He has delivered guest lectures at the University of Minnesota, School of Medicine, Albany Medical College, the University of Virginia School of Medicine, and others. He was an invited participant at the International Symposium on "Biogenic Amines and Their Enzymes in the Neuropathology of Schizophrenia" held in Strasbourg, France, presenting a paper on "Behavioral Effects of a New Dopamine-beta-hydroxylase Inhibitor (Fusaric Acid) in Man". He also presented an invited paper on the "Mechanisms of Action of Lithium in Manic-Depressive Patients" at the International Symposium on Lithium, Quebec City, Canada.

At the Annual Meeting of the American Psychiatric Association (APA), Dr. Goodwin will present a paper on "Central Norepinephrine Metabolism in Affective Illness: Studies with MHPG in the Cerebrospinal Fluid". In addition he will participate in the APA meeting as co-author of five other papers. Dr. Goodwin was Guest Lecturer at a symposium on Psychopharmacology, sponsored by the Ohio Psychiatric Society, and delivered a lecture on the biochemical basis of mental illness under the sponsorship for the Foundation for Medical Education of Washington, D.C.

Dr. Greenwood was an invited lecturer at the Department of Psychiatry, Medical College of Virginia, Richmond, Virginia. At the Annual Meeting of the American Psychiatric Association (APA), Detroit, Michigan, he will present a paper entitled "Drugs and Psychodynamic Shifts in Affective Illness".

Dr. Sack: See Project Report No. M-CS-Ps(C)-19.

Publications:

Goodwin, F.K. and Bunney, W.E., Jr.: Psychobiological Aspects of Stress and Affective Illness. In Separation and Depression, Senay, E. (Eds.), American Association for the Advancement of Science, pp. 91-112, 1973.

Kotin, J. and Goodwin, F.K.: Depression during mania: clinical observations and theoretical implications. Amer. J. Psychiat., 129:679-686, 1972.

Goodwin, F.K. and Murphy, D.L.: Biological Factors in the Affective Disorders and Schizophrenia. In Psychopharmacological Agents, Gordon, M. (Eds.), Vol. III, pp. 5-26, Academic Press, 1974.

Kotin, J., Post, R.M. and Goodwin, F.K.: Drug treatment of depressed patients referred for hospitalization. Amer. J. Psychiat., 130:1139-1141, 1973.

Post, R.M. and Goodwin, F.K.: Simulated behavior states: an approach to specificity in psychobiological research. (A.E. Bennett Award Paper). Biol. Psychiat., 7:237-254, 1973.

Gershon, E.S., Dunner, D.L., Sturt, L., and Goodwin, F.K.: Assortative mating in the affective disorders. Biol. Psychiat., 7:63-74, 1973.

Goodwin, F.K. and Sack, R.L.: Affective disorders: the catecholamine hypothesis revisited. In Frontiers of Catecholamine Research, Usdin E. and Snyder, S. (Eds.), pp. 1157-1164, Pergamon Press, 1973.

Gillin, J.C., Post, R.M., Wyatt, R.J., Goodwin, F.K., Snyder, F., and Bunney, W.E., Jr.: REM inhibitory effects of L-DOPA infusion in depressed patients. Electroenceph. Clin. Neurophysiol., 35:181-186, 1973.

Buchsbaum, M., Landau, S., Murphy, D.L., and Goodwin, F.K.: Average evoked response in bipolar and unipolar affective disorders: relationship to sex, age of onset, and monoamine oxidase. Biol. Psychiat., 7:199-212, 1973.

Ablon, S.L. and Goodwin, F.K.: High frequency of dysphoric reactions to tetrahydrocannabinol among depressed patients. Amer. J. Psychiat., 131:448-453, 1974.

Carlson, G.A., Kotin, J., Davenport, Y.B., and Adland, M.: Follow-up of 53 bipolar manic-depressive patients. Brit. J. Psychiat., 124:134-139, 1974.

Post, R.M., Kotin, J., and Goodwin, F.K.: Cocaine: clinical effects in depressed patients. Amer. J. Psychiat., 131:511-517, 1974.

Serial No. M-P-C-(C)-12
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of heredity and environment in schizophrenia.

Previous Serial Number: Same.

Principal Investigator: David Rosenthal.

Other Investigators: Paul H. Wender (University of Utah), Seymour S. Kety (Harvard), Shmuel Nagler (Israel), Fini Schulsinger Denmark).

Cooperating Units: None.

Man Years:

Total:	7.0
Professional:	3.0
Others:	4.0

Project Description:

Objectives: To understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

Methods Employed: 1. A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. 2. An intensive multidisciplinary study of a family with one-egg quadruplet daughters concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. 3. Studies of adoptees and their biological and adoptive families. 4. A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

Major Findings: 1. The analysis of the literature has been reported in a series of papers and in the study of the quadruplets. A textbook which organizes and explicates the literature on the genetics of behavioral disorders, and a modified version of this book have been published. 2. Several papers on our adoption studies are now published. The studies indicate that hereditary factors contribute to the development of schizophrenia, and to other disorders that we include in a group called the schizophrenia spectrum. Several articles are in preparation and eventually a monograph will be written on the adoption studies. Data analysis is continuing. 3. One hundred subjects in

the Israel study have been examined, and the research findings are currently being analyzed and evaluated. Preliminary findings indicate that offspring of schizophrenics have more neuropathological signs in childhood than do controls. Two subjects have had a breakdown and one is on the verge of breakdown. All three have a schizophrenic parent.

Significance to Bio-medical Research and the Program of the Institute: We have resolved a chronic, critical problem in psychiatry by demonstrating beyond any reasonable doubt that genetic factors are importantly involved in the etiology of schizophrenia and related mental disorders. Moreover, the evidence thus far suggests that the mode of genetic transmission is polygenic or a dominant single gene with polygenic modifiers.

Proposed Course of Project: We are examining additional subjects in Denmark with the following goals in mind: 1. to try to discriminate gene carriers and non-gene carriers with respect to personality and test variables; 2. to compare the fate of gene-carriers who are reared in the parental home as compared to those reared in adoptive homes; 3. to compare Ss who have a psychotic biological parent but who are reared by nonpsychotics with Ss whose biological parents are not psychotic but who are reared by a psychotic adoptive parent; 4. to find the incidence of schizophrenia spectrum disorder in a random sample of the Danish population. In Israel, our goal is to prepare a volume on our findings and their theoretical implications. During this year we are carrying out reexaminations of this unique sample of subjects.

Honors and Awards: None.

Publications:

Rosenthal, D.: Review of A. R. Kaplan, Genetic factors in "Schizophrenia." J. Nerv. Ment. Dis., 157: 224-227, 1973.

Rosenthal, D.: Studies in Denmark of heredity and schizophrenic disorders. In B. S. Brown, & E. Fuller Torrey (Eds.), International collaboration in mental health. U.S. D.H.E.W., NIMH, Publication No. (HSM) 73-9120. Washington, D.C., U.S. Govt. Print. Off., 106-112, 1973.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Reaction time in schizophrenia.

Previous Serial Number: Same

Principal Investigator: Theodore P. Zahn

Other Investigator: Paul Fedio

Cooperating Unit: Surgical Neurology Branch, NINDS

Man Years:

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objectives: To study deficits in attention in schizophrenia, particularly as manifested in difficulties in adopting and maintaining preparatory sets. To study the specificity of such deficits to schizophrenia. To study possible organic and genetic determinants of such deficits. To determine the relation between such deficits and the severity of schizophrenic symptomatology.

Methods Employed: (A) A reaction time (RT) technique which puts severe demands on attention and which discriminates well between schizophrenic and non-schizophrenic subjects is being given to patients before and after unilateral temporal lobectomy for the relief of psychomotor epilepsy. This procedure and a choice technique which compared RT to monaural stimulation in each ear and responses with the same or opposite hand have also been used on postoperative temporal lobe followup cases and normal controls.

(B) Acute schizophrenic and non-schizophrenic patients are being tested with these procedures soon after admission, on discharge from the Clinical Center after 3-4 months and on a followup 6 months to 1 year later. The results will be compared with intensive symptom ratings.

Major Findings: We continue to find that about half of the temporal lobe followup cases give essentially normal performance despite some apparent personality disturbance. Most patients tested 10 - 15 days postoperatively have shown a decline from their preoperative performance level, suggesting a possible recovery of function over time. The followup cases are also slower in choice RT but further analysis of the data is needed to determine if this slowing is disproportionate to the simple RT level.

Significance to Biomedical Research and the Program of the Institute: These studies are designed in part to explore the possibility of a biological basis for schizophrenia. Since patients with lesions in the temporal lobe frequently exhibit a schizophrenic-like personality picture and since patients with other types of brain lesions have been found to exhibit reaction time deficits (but of different types than those shown by schizophrenics) the reaction time performance of these patients might indicate if a temporal lobe disturbance is possibly involved in schizophrenia. The reaction time studies with schizophrenics add to our knowledge of the specific psychological processes involved in the disorders of set and attention that seem to be important aspects of the schizophrenia process.

Proposed Course of Project: Further collection of data on followup temporal lobe cases is in progress. When enough data have been collected the effects of the side of the lesion and correlations with the amount of tissue removed can be determined. Further data collection on schizophrenic and non-schizophrenic patients.

Honors and Awards: None.

Publications: None

Serial No. M-P-C(C) - #17
1. Laboratory of Psychology
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychophysiological Responsivity in Schizophrenia.

Previous Serial Number: Same

Principal Investigator: Theodore P. Zahn

Other Investigators: David Rosenthal, William Pollin, Loren Mosher, Carmi Schooler

Cooperating Units: Section on Twin and Sibling Studies, APB, NIMH
Section on Technical Development, MHIRP
Section on Psychiatric Assessment, APB, NIMH

Man Years:

Total:	.5
Professional:	.2
Other:	.3

Project Description:

Objectives: The general purpose of these studies is to investigate schizophrenic-normal differences in the relative autonomic responsivity to various stimuli, the relationship of autonomic activity to adequacy of performance and to psychiatric condition, and possible genetic determinants.

Methods Employed:

1. Peripheral measures of autonomic functioning, namely GSR, heart rate, finger pulse volume, respiration and skin temperature are recorded during several sessions in which stimuli are presented and tasks are performed which vary in the demands placed on the subjects: no stimuli, simple auditory stimuli with no response required, the same with a "casual" response required, reaction time, word association, mental arithmetic, and cold presser. Specific and non-specific response frequencies and amplitudes, baseline values and baseline changes are measured. These procedures have been used with identical twins discordant or concordant for schizophrenia and their parents and with the adoptive and biological parents of schizophrenic patients in order to see if whatever genetic influence there is in schizophrenia is manifested in autonomic functioning.

Currently, acute schizophrenic and non-schizophrenic patients are being tested on a shorter version of the above procedures soon after admission, at discharge from the Clinical Center (3-4 months later) and at a 6 month to 1 year followup.

Methods have been developed in our laboratory to analyze skin conductance, heart rate, skin temperature and finger pulse volume data automatically from taped analog records by means of the SEL 810B computer.

2. Two new experimental tasks are being developed with preliminary work on normal controls. One is a two flash threshold determination using a forced choice technique. There are data in the literature suggesting that two flash threshold varies with arousal level and that this relationship is different for schizophrenic and normal subjects. The other new procedure is a tachistoscopic recognition procedure, also using a forced choice technique, which lends itself to concurrent psychophysiological recording. With this technique we hope to be able to test the differential psychophysiological responsiveness of schizophrenics and normals to such things as task difficulty, success and failure, positive and negative reinforcements, etc. as well as being able to separate sensory reception aspects from response aspects of stimulu complex.

Major Findings: 1. Previously we had found that the differences between the schizophrenic and non-schizophrenic members of the twin pairs were similar to the differences between unrelated schizophrenic and normal subjects in that: (a) despite equal autonomic responsivity to meaningless stimuli, the schizophrenics were significantly hyporeactive to more meaningful stimuli; and (b) the schizophrenics showed higher arousal levels under nondemanding conditions but the controls showed a greater increase in arousal under the mild stress of task performance. The latter group, in fact, showed some tendency to be hyperreactive to meaningful and/or stressful stimuli compared to normal subjects who do not have a familial history of schizophrenia.

2. Preliminary impressions of the data currently being collected on acute schizophrenics suggest that when the patients are exhibiting acute schizophrenic symptomatology, they show the hyporeactivity to meaningful stimuli as described above, but become markedly more reactive, perhaps hyperreactive, during periods of remission from the schizophrenic symptoms.

3. Preliminary results from the correlational analyses show unexpected but consistent negative correlations between electrodermal and heart rate indices of arousal and urinary measures of the precursors and metabolites of epinephrine and norepinephrine (dopamine, metanephrine, and normetanephrine). Correlations among the autonomic performance and behavioral variables suggest that schizophrenics with high resting (non-specific) arousal levels have poorer performance and more severe symptomatology than those with lower arousal levels, while the relationships of task produced increments in arousal have just the opposite relationships with the behavioral variables. Similar, but much less marked relationships are found in controls.

4. Preliminary analyses of the genetic determination of psychophysiological variables have shown that overall autonomic arousal levels, variability and responsivity seem to have a significant genetic-familial

component, while performance measures and the autonomic response to demand do not show appreciable genetic influence.

5. A review of the literature on the relationships of autonomic phenomena to behavior in schizophrenia has revealed much support for the implication from our research that there is no generalized deficit in autonomic reactivity in schizophrenia, but that such deficits occur specificially to meaningful or demanding stimuli such as those involved in task performance. It is also apparent from the literature that a simple inverted U relation between autonomic arousal and performance is not well confirmed, and that what we have called specific and non-specific arousal might have different relationships to behavior in schizophrenia.

Significance to Biomedical Research and the Program of the Institute: The role of autonomic arousal and responsivity in schizophrenic deficits in behavior remains obscure, but it is clear from our own research and that of other investigators that a low arousal level is not the critical factor as has sometimes been postulated. Our evidence suggests that a high arousal level that is non-task-related (non-specific arousal) and low task-related increments in arousal or the relationship between these may be related to such performance deficits. Further elucidation of the autonomic concomitants of symptoms and behavior deficits in schizophrenia may give us an increased understanding of the biological aspects of this condition and therefore may lead to more specific therapeutic procedures.

In view of the abundant evidence that genetic factors are of importance in the etiology of schizophrenia, it is reasonable to ask if a "schizophrenia genotype" is manifested in the autonomic nervous system. Some suggestions of autonomic hyperactivity in our co-twins indicate that if there is a genetically determined autonomic pattern that predisposes individuals to schizophrenia it is of a markedly different nature than that shown by individuals who are, in fact, schizophrenic. This conclusion receives some indirect support from our impression that subjects who have remitted from an acute schizophrenic episode seem to show a similar pattern of autonomic response as the non-schizophrenic co-twins.

Proposed Course of Project: 1. Further analysis of the data from the twins and their families will include (a) examining more autonomic variables such as vasomotor changes, and other aspects of electrodermal functioning based on new findings in the literature; (b) multivariate statistical analysis of group differences; and (c) partial correlations on selected subsets of the large set of variables from different investigators.

2. Testing of patients on 4E will continue until the current project on that ward is terminated this summer and the data will be analyzed. Hopefully followup testing will be continued.

3. Continued exploration of the utility of the two-flash and recognition techniques.

Honors and Awards: None

Publications: Zahn, T. P.: Psychophysiological concomitants of task performance in schizophrenics. In: Zubin, J., Sutton, S., and Keitzman, M. (Eds.) Experimental Approaches to Psychopathology. Academic Press. In press.

Serial No. M-P-C-(C) -36
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS - ADAMHA - NIMH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Psychological Correlates of Cortical Evoked Responses.

Previous Serial Number: M-P-C-(C) - 36, 44

Principal Investigator: Monte Buchsbaum, M.D.

Other Investigators: Thomas Bittker, M.D.; Thomas Chase, M.D.; Frederick Goodwin, M.D.; Robert Henkin, M.D., Stephen Landau, M.D.; John Molino, M.D.; Dennis Murphy, M.D.; and Redford Williams, M.D.

Cooperating Units: Adult Psychiatry Branch, NIMH
Laboratory of Clinical Science, NIMH
Clinical Endocrinology, NHLI
National Bureau of Standards

Man Years:

Total:	2.2
Professional:	1.2
Other:	1.0

Objectives: Exploration of relationships between perceptual response patterns, psychiatric clinical dimensions and neurophysiological measures; the development of improved techniques for collecting and analyzing neurophysiological data collected from intact human subjects; detailed study of the interaction between attention, cortical area of recording and individual differences in neurophysiological response.

Methods Employed: Research in this project focuses on understanding the psychophysiology of perception and thus providing a background for studies of perceptual and cognitive disturbances in psychiatric patients. Its work is closely coordinated with clinical research in the section on Perceptual and Cognitive Studies, Adult Psychiatry Branch.

Central nervous system function has been difficult to study in psychiatric patients by either conventional neuroanatomic or implanted electrode techniques, as the former requires a dead patient (or an animal model of the disease) and the latter

requires a hazardous surgical procedure. Obviously, neither method permits long-term study of psychiatric episodes. With the development of average evoked response techniques, which allow recovery of relatively specific CNS signals from scalp EEG, neurophysiological attempts to study psychiatric disorders received new impetus. The average evoked response (AER) is a particularly promising tool for studying perception in the psychiatric patient because, unlike traditional psychophysical techniques, it does not depend upon the patient's report or overt performance. Thus, the importance of motivation or motor activity is minimized and a clearer separation of perceptual (input) and performance (output) factors may be achieved.

The development of computer technology has been an essential step in the application of average evoked response measures to large scale patient testing. During the past year, our system for collection and processing of the evoked response data has been significantly expanded. Through the use of existing SEL computer hardware and a new A/D converter built by the Section on Technical Development, together with ingenious new software, we can now run two high speed psychophysiological experiments simultaneously. This has made the scheduling of clinical experiments much more convenient and reliable. Addition of the fixed head disk to the SEL has also made possible significant increases in other programs including a data management system, and other real-time programs.

Major Findings: Coping with sensory overload is perhaps the major perceptual problem of 20th century urban man; individuals differ widely both in their relative success in meeting the challenge and in their styles of coping. Evoked responses to loud, repetitious, meaningless and meaningful stimuli have been studied in subjects attending to or trying to ignore the stimuli. The early discovery that some subjects paradoxically showed actual decreases in AER amplitude with increasing stimulus intensity led to the development of a perceptual style concept known as "stimulus intensity control," and to a variety of clinical and more basic investigations.

1. Pain and Noise Studies. Based on the results of several studies we have viewed evoked response augmentation (increasing amplitude with increasing stimulus intensity) as indicative of a tendency to increase the perceived intensity of stimuli, and to be relatively insensitive to extremely weak stimuli. Evoked response reducing (decreasing amplitude with increasing stimulus intensity), in contrast, is associated with a tendency to decrease the perceived intensity, to avoid exciting external stimulation and to be relatively sensitive to extremely weak stimuli. Recently, two new experimental results

have given the augmentation/reduction formulation further construct validity.

a) Noise Tolerance Studies (in collaboration with Dr. John Molino, NBS). Individual differences in noise tolerance and auditory AER were studied in 27 adult volunteers. Noise tolerance was assessed at the NBS by measuring the rate at which subjects pressed a key to decrease noise while attempting to use a teaching machine. In a separate session at NIMH, AERs to four intensities of noise were presented. Individuals who were tolerant of noise were indeed reducers on the noise AER. Individual differences in AER attentional effects were also significant predictors of noise tolerance.

b) Pain Stimulation Studies. Thirty adult volunteers were studied using electrical stimuli delivered to the forearm via a Tursky electrode. Subjects rated the stimuli on a four point subjective scale. After the rating trials, AER were recorded to the same electrical stimuli from vertex and central EEG leads. Individuals whose ratings indicated they were relatively pain tolerant showed AER which decreased in amplitude over 16 milliamperes (i.e., reducers). AER from low intensity stimuli showed no difference between pain tolerant and pain intolerant individuals' mitigating against skin impedance, pain or other nonspecific factors being the source of group differences. Individual differences in pain tolerance were also associated with differences in AER habituation and interstimulus interval effects, especially at high intensities.

2. Insomnia and Sensory Stimulation. Eighteen chronic insomniacs, whose sleep problems were confirmed by all night EEG recordings were matched with normal sleepers on age, sex and education. The insomniacs differed from normal sleepers on the following dimensions: 1) insomniacs were significantly more depressed on Zung's Depression Scale and the MMPI D. Secondly, insomniacs showed more anxious worrying behavior. Thirdly, insomniacs appeared to be sensory reducers, as evidenced by lower AER responses to sound at high intensities and by low scores on Zuckerman's Sensation Seeking Scale. A step-wise regression analysis revealed that the sensory reducing dimension accounted for the most variance in predicting sleep efficiency as measured by EEG criteria.

3. Psychopathology of Attention and Arousal. Differences between schizophrenics and normals on psychophysiological measures have been credited both to "attention" and "arousal" regulation defects. Most measures have been relatively nonspecific, however, and open to many interpretations. A number of investigators have reported that average evoked response (AER) amplitude increases when attention is toward the

evoking stimulus -- most clearly perhaps for the N140 component. Based on psychophysical studies indicating that right visual field for stimulus presentation bettered attentional performance and our finding that dim stimuli showed more prominent attentional effects than bright ones, we investigated cortical laterality for attention. We presented bright and dim flashes of light to the left and right hemifields and recorded AER bilaterally from both occipital and tempero-preoccipital leads (Wernicke's area or W). Attentional amplitude increases were two to four times as great for N140 in AER to dim flashes prnsented to the right visual field (left hemiretina) and recorded from the left W lead as for any other combination. This high specificity suggests the importance of Wernicke's area for attentional processes. Several recent psychophysiological studies by Venables have pointed toward lateralized deficits in schizophrenia; a neuroanatomic study by Rosenthal and Bigelow at St. Elizabeths Hospital has found corpus callosum abnormalities in schizophrenics. Taken together, these and other findings suggest the value of laterality and attention studies in schizophrenic patients.

Individual differences in the amount of AER amplitude change with attentional deployment are prominent even in normal individuals. Based on relationships between peripheral vascular resistance, sensory intake behavior and catecholamine levels, we hypothesized that dopamine-B-hydroxylase (DBH) might be a predictor of these differences. In two studies, DBH levels correlated significantly with the amount of amplitude increase in AER for N140 when subjects counted stimuli as opposed to doing mental arithmetic. In a third study, uninstructed subjects' AER amplitudes correlated highly with DBH. This is intriguing in view of the finding that schizophrenics, often exhibiting attentional deficits, have also been implicated as having low brain DBH (although the relationship between central and peripheral DBH is not well understood).

4. Biochemical and Clinical Studies. These studies are reviewed in M-AP(C) - 21-2, "Studies of Perceptual and Cognitive Styles" in Psychiatric and Non-psychiatric Subjects.

5. Twin Studies. Average evoked responses, perceptual tasks, autonomic psychophysiology and interview material have been collected in 60 normal twin pairs. Detailed analysis of visual amplitude/intensity data was reported last year. High heritability was found for the augmenter-reducer patterns of response. Data on AER evoked from sinusoidally-modulated light at four different levels of modulation revea'ed a similar pattern of heritability for depth of modulation/intensity slopes. Also of interest was the finding of heritability of

hemispheric asymmetry and of hemispheric "mirror imaging" in Mz twins but not in Dz twins.

A negative finding of some interest is the low heritability of the N140 component. This component appears to be an attentional correlate in other studies. Since subjects were not given a specific task to do during AER recording, non-genetic attentional differences between twins might affect this component. This appears consistent with Rosenthal's finding that attentional measures (reaction time) followed rearing rather than biological parental patterns.

6. Individual Differences and Reliability of Information Transmission in Absolute Judgments of Loudness, Brightness and Line Lengths. By drawing an analogy between the human observer and a communication channel, it is possible to apply principles derived from information theory to a variety of psychophysical problems. Because of recent reports purporting to show diminished "information transmission" in schizophrenics, studies of the reliability of information transmission measures were done in normal subjects.

Information transmission, as estimated from absolute judgments of loudness, brightness and line length, was measured in 35 normal subjects. Each subject was tested on all modalities on each of three days. Individuals varied widely in their ability to transmit information and were consistent across modalities and days; reliabilities for loudness, brightness and line length between days 2 and 3 were 0.72, 0.81 and 0.89, respectively. The mean intercorrelation between modalities was 0.40. WAIS digit span but no other intelligence scale was positively correlated with information transmission. The results suggest the existence of a single information processing facility in the central nervous system. AER data collected during an identical task clearly indicated that "reducing" was not associated with poor perceptual performance, but on the contrary with veridical responses.

7. Signal Processing Studies. Several investigators have reported that AER appear more variable in schizophrenic than in normal subjects. This apparent variability could result from the schizophrenic having a small signal buried in background noise (indeed suggested by our finding of reducing) or from variability of the signal itself. A major theoretical issue associated with evoked response research has been to adequately describe the signal plus noise relationship in a manner that allows for quantification of signal variability and other measures of underlying mechanisms. New methods of signal analysis are being explored with these aims in mind. These methods include sample correlation techniques, phase spectra

averaging, and other signal decomposition techniques. It is hoped that with further development of these methods, measures of response variability and nervous system "noise" that can be utilized in clinical populations will result. Hopefully this will also lead to a better understanding of stimulus intensity/amplitude factors and thus allow for a better differentiation of clinical and normal groups.

Significance to Biomedical Research and the Program of the Institute:

This project, in helping to give a neurophysiological basis to psychiatric diagnosis and prognosis, contributes to the effort to understand the mechanisms of psychiatric disease.

Proposed Course of Project:

1. Clinical and biochemical studies are summarized in project M-AP(C)-21-2.

2. Psychopathology of Attention. Schizophrenic, bipolar and unipolar affective disorder patients will continue to be tested using hemiretinal stimulation with multiple bilateral EEG leads.

3. Pain and Sensitivity Studies. Continued studies of biochemical correlates of pain response (urinary MHPG) and the AER are underway with Dr. Goodwin. Data on 22 new subjects given electrical shock AER while urine was collected are being analyzed; patient studies are also underway. Instrumentation to use somewhat more as well as less intense auditory (40-100 dB) and visual stimuli (0.01 to 1000 foot lamberts) has been purchased.

4. Mathematical studies of signal variability. Empirical tests of several new models are currently underway and work will continue.

5. Habituation Studies. We are continuing to analyze data from a large parametric study examining the interaction of stimulus intensity, habituation and intensity. The augmenter-reducer dimension appears to interact strongly with habituation; augmentation being associated with "sensitization" or the absence of habituation at high intensities. Complex factors involved may be further resolved by experiments using the expanded stimulus intensity range to become available shortly.

Publications:

Gillin, J. C., Jacobs, L. S., Fram, D. H., Williams, R., Buchsbaum, M., and Snyder, F.: Partial REM phase deprivation and schizophrenia: An experimental reappraisal. Arch. Gen. Psychiat. In press.

Buchsbaum, M.: Cybernetics and the cortical evoked potential. Cybernetics Technique in Brain Research and the Educational Process, 1971, Fall Conf. pp. 69-77.

Schechter, G. and Buchsbaum, M.: The effects of attention stimulus intensity and individual differences on the average evoked response. Psychophysiology. 10 (4): 392-400, 1973.

Buchsbaum, M. and Coppola, R.: Computer use in bioelectric data collection and analysis. In: Bioelectric Recording Techniques (Eds) Thompson, R. and Patterson, D., Academic Press, New York, pp. 299-241, 1974.

Buchsbaum, M.: Variability, intensity and laterality effects in AER in Mz and Dz Twins. Psychophysiology. 11 (2): 226-227, 1974.

Buchsbaum, M., Henkin, R. I., and Christiansen, R. L.: Age and sex differences in averaged evoked responses in a normal population, with observations on patients with gonadal dysgenesis. Electroenceph. Clin. Neurophysiol. In press.

Buchsbaum, M., Copolla, R., and Bittker, T. E.: Differential effects of "congruence," stimulus meaning, and information on early and late components of the average evoked response. Neuropsychologia. In press.

Landau, S. G., Buchsbaum, M., Coppola, R., Sihvonen, M.: Individual differences and reliability of information transmission in absolute judgments of loudness, brightness and line lengths. Perceptual and Motor Skills. In press.

Coursey, R. D., Frankel, B. L., and Buchsbaum, M.: Evoked response differences between chronic insomniacs and normal sleepers. Sleep Research, Vol. 3, 1974. In press.

Serial No. M-P-C-(C)-39
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of heredity and environmental factors in schizophrenia.

Previous Serial Number: Same

Principal Investigator: Paul H. Wender

Other Investigators: David Rosenthal, John Rainer (New York)
Laurence Greenhill

Cooperating Units: Research Foundation for Mental Hygiene, Inc.,
722 West 168th Street, New York, N.Y. 10032

Man Years:

Total:	4.1
Professional:	3.7
Other:	.4

This project is discontinued.

Serial No. M-P-C-(C)-40
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Changes in neurological and psychological functioning in children with minimal brain dysfunction receiving d-amphetamine.

Previous Serial Number: Same

Principal Investigator: Paul H. Wender

Other Investigators: Monte Buchsbaum

Cooperating Units: None

Man Years:

Total	1.1
Professional:	1.1
Other:	0

This project is discontinued.

Serial No. M-P-C-(C)-43
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Lithium as a therapeutic agent in hyperkinetic behavior disorders of childhood.

Previous Serial Number: Same

Principal Investigator: Paul H. Wender

Other Investigators: Ronald O. Rieder, James Weiss, Monte Buchsbaum,

Cooperating Units: Office of the Chief, Laboratory of Clinical Science

Work in this project is temporarily suspended and is being held in abeyance.

Serial No. M-P-C-(C)-45
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The offspring of schizophrenics: markers of a schizophrenic disposition.

Previous Serial Number: None

Principal Investigator: Ronald O. Rieder

Other Investigators: David Rosenthal, Paul Wender

Cooperating Units: PHS-NIH-NINDS-PRB

Man Year:

Total:	4.2
Professional:	2.4
Other:	1.8

Project Description:

Objectives: To discover differences between the offspring of schizophrenics and controls, at an early stage in life, which may represent a predisposition to develop schizophrenia later in life.

Methods Employed: This study has utilized the data previously collected by the National Institute of Neurological Disease and Stroke, Perinatal Research Branch, in their "Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood." In that project, some 55,000 pregnancies and the offspring were studied, with the 7-year follow-up now just completed. About 900 of the mothers or fathers had had a psychiatric hospitalization, and of this group, we took 220 who were studied in the Boston area to use for our study. We gathered the records of their psychiatric hospitalizations, and we proceeded to diagnose these subjects. We diagnosed 150 parents, and assigned a diagnosis of schizophrenia to 67 of these. The 150 families produced a total of 210 children who had been part of the NINDS study, and 93 of these were the offspring of schizophrenics. Taking the families containing a schizophrenic parent, we selected a matched control group, and began comparing the findings for our two groups on the investigations which the NINDS-PRB had performed.

Major Findings: There is an increased rate of fetal and neonatal death among the offspring of schizophrenics as compared with controls. Some of these deaths were associated with neurological malformations. Reasons for the deaths were not found at autopsy. The deaths were among the offspring of parents with chronic and severe forms of schizophrenia, as opposed to the acute or borderline forms. The deaths occurred predominately among those offspring who had two schizophrenic parents, or had a schizophrenic mother, rather than among those who had a schizophrenic father only. Two comparison groups, offspring of possible schizophrenics and offspring of non-schizophrenic patients, did not show an increased rate of fetal or neonatal deaths when compared to their matched controls.

There is no difference in birth weight or Apgar score between the offspring of schizophrenics and controls.

Significance to Bio-medical Research and the Program of the Institute: This work follows the work of this Laboratory on the genetics of schizophrenia. We need to find how the genetic factor underlying schizophrenia clinically manifests itself if we are to effectively intervene or do biochemical and physiological investigations of the development of schizophrenia.

Proposed Course of the Project: During the next year we will continue to investigate the data which has been collected on these offspring of schizophrenics. This includes serial neurological exams, psychological exams, and language exams.

Honors and Awards: None

Publications: The offspring of schizophrenics: fetal and neonatal deaths. Submitted to the Archives of General Psychiatry.

Serial No. M-P-C(C) - #46
1. Laboratory of Psychology
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychophysiological Changes During the Menstrual Cycle.

Project Serial Number: M-P-C-(C) - #17

Principal Investigator: Theodore P. Zahn, Ph.D.

Other Investigators: Betsy Little (Guest Worker) Raymond Matta, Friedhelm Lamprecht

Cooperating Units: Laboratory of Clinical Science

Man Years:

Total:	.6
Professional:	.1
Other:	.5

Project Description:

Objectives: To study autonomic changes through the menstrual cycle. To study the relationships between autonomic changes and changes in time estimation, reaction time, and mood throughout the menstrual cycle. To elucidate the mechanisms responsible for the physiological changes seen in women, progesterone was administered to men and dopamine-beta hydroxylase activity has been assessed in women.

Method: For women, a short experimental procedure was used every day for each subject for a complete cycle. Five autonomic measures were used (finger pulse volume, heart-rate, respiration, skin temperature and skin resistance) in three situations:

(i) giving the subject moderate intensity tones to listen to
(ii) asking the subject to estimate 5-sec. intervals
(iii) giving the subject a simple reaction-time task where she had to lift a key as quickly as possible on hearing a tone.

For men, the same testing procedure was used as for women. They were tested four days a week for four weeks. During one week (double blind) they received 50 mgm of progesterone per day. During the remaining three weeks they received a placebo.

Both subjects and experimenter filled out a mood scale daily.

In a third study the course of plasma dopamine-beta hydroxylase (DBH) activity was assessed daily in six women over the course of a complete cycle.

Major Findings: Several psychophysiological and mood variables showed statistically significant changes over the course of the menstrual cycle. Heart rate and respiration rate both reached a peak late in the luteal phase. Skin conductance reached its lowest point in the luteal phase but a few days earlier than the heart rate peak, coinciding more with the peak body temperature. Heart rate variability peaked at ovulation and was low in the luteal phase. Autonomic responsivity to the stimuli of the daily task peaked just before ovulation, and on some of these variables, particularly the responses to the more "demanding" reaction time task, there was a second peak during the luteal phase. A mood dimension of "postive activation" describing feelings of vigor, well-being and affection reached a peak at ovulation and was lowest before and during menstruation.

The administration of progesterone in men was accompanied by an increase in body temperature a decline in skin conductance level, an increase in the "sluggishness" of electrodermal responses manifested in increased rates of rise and recovery, a decrease in heart rate variability and increase in reaction time. These effects, except for the temperature rise persisted for several days to a week after withdrawal of the progesterone. Expected changes in heart and respiratory rates and in mood did not occur although several subjects reported fatigue after progesterone withdrawal. Thus the data only partially support the hypothesis that increased progesterone levels are responsible for the psychophysiological and mood changes occurring in women premenstrually.

Plasma DBH activity increased during the follicular phase of the cycle reaching a peak soon after ovulation and decreased to a minimum during the premenstruum. The activity of this enzyme does not seem to parallel the levels of estrogen or progesterone nor those of the psychophysiological variables, but the mood "Surgency" showed comparable changes over the cycle.

The data from these studies are being analyzed to find the day-to-day relationships between mood and autonomic activity and to assess moderately long term adaptation to testing procedures. Preliminary findings suggest that fatigue has a damping effect on autonomic activity and that there is considerable long term (at least 3 weeks) continuing adaptation manifested on many variables.

Significance to Biomedical Research and the Program of the Institute:
Previous studies have shown that women are statistically more likely to have accidents, commit crimes and suffer exacerbations of psychiatric symptoms during premenstrual periods. These studies were undertaken to throw light on the biological basis for these and related phenomena. Although we found reliable autonomic changes over the cycle in normal women, practically none of the variables were at an extreme value during the immediate premenstruum. Rather, the most extreme values were usually seen either around the time of ovulation, coinciding with a peak in feelings of positive activation, or in the luteal phase a week or so premenstrually. Since many variables were changing rapidly during the premenstrual period and since in the study of progesterone effects in men, similar changes in

some variables were seen after withdrawal of progesterone it is suggested that premenstrual symptoms may be due to the sudden biological readjustments consequent on progesterone withdrawal. However, the studies also show that other factors besides progesterone, probably estrogen, are important.

Proposed Course of Project: A logical further study would be to test the effects of estrogen and perhaps combined estrogen and progesterone in males. However, our unit has insufficient personnel to attempt this at present.

These studies have generated a wealth of data on the day-to-day covariations in mood self-ratings and autonomic variables as well as in covariations among various psychophysiological variables and their adaptation over time. Analyses of these aspects of the data will continue. A paper on the relations between mood and autonomic variables has been accepted for presentation at the British Psychological Association meetings this year.

Honors and Awards: None

Publications: Little, B. C., and Zahn, T. P.: Changes in mood and autonomic functioning during the menstrual cycle. Psychophysiology, in press.

Little, B., Matte, R. J., and Zahn, T. P.: Physiological and psychological effects of progesterone in man. J. Nerv. Ment. Dis., in press.

Lamprecht, F., Matta, R. J., Little, B. C., and Zahn, T. P.: Plasma dopamine-beta-hydroxylase (DBH) activity during the menstrual cycle. Psychosomatic Med., in press.

Serial No. M-P-C(C) - #47
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychophysiological Concomitants of Minimal Brain Dysfunction in Children.

Previous Serial Number: M-P-C(C) - #17

Principal Investigator: Theodore P. Zahn

Other Investigators: Paul H. Wender, Betsy Little

Cooperating Units: None

Man Years:

Total:	1.9
Professional:	.8
Other:	1.1

Project Description:

Objectives: To compare MBD children with normal children in autonomic functioning. To assess the effects of drug therapy. To predict MBD children's response to drug therapy.

Methods Employed: Peripheral measures of autonomic functioning, namely skin resistance, heart rate, respiration, skin temperature, and pupil size are recorded during two sessions in which children are exposed to mild auditory and visual stimuli, perform reaction time and rod and frame tests and perform a cold pressor procedure. Parents are given questionnaires relating to child rearing attitudes and practices, and their child's behavior.

Subjects are children from the Washington area with a diagnosis of MBD under treatment by a psychiatrist. Controls are from a nearby elementary school and have been chosen by their teachers as not having significant behavior problems. The patients are tested both on and off therapy with stimulant drugs (amphetamine and methylphenidate).

A small number of MBD patients who did not respond to amphetamines were given a clinical trial of lithium. Those subjects have been tested both on drugs and off drugs.

Major Findings: Lithium produced an increase in heart rate and respiration rate in most subjects.

Comparison of MBD children on the first session off drugs with control children showed no significant differences in the base arousal indices of heart rate, skin conductance, skin temperature or frequency of non-specific electrodermal responses. However the electrodermal responses of the MBD children did have longer recovery times, a measure thought by some investigators to reflect arousal level. The most striking psychophysiological difference between the groups was a general sluggishness of autonomic activity, manifested by electrodermal responses (both spontaneous and elicited by stimuli) which took longer to reach a peak value and, as mentioned above, to recover. Heart rate responses also showed this pattern but a lesser extent.

In a second experimental session it was found that pupillary and heart rate responses to light flashes did not differentiate the groups, but the MBD children were significantly less responsive on these measures to the stimuli of a reaction time test as compared to controls. In this session, which was somewhat more stimulating than the first session, the MBD children had higher electrodermal and pupillary indices of arousal.

Stimulant drugs, which improved the children's home and school behavior as judged by parental and psychiatrists ratings, also improved attention as estimated by reaction time performance, and produced increases in the autonomic arousal indices of heart rate, skin temperature, skin conductance and pupil size. The drugs also produced a marked reduction in electrodermal responsivity. However, when the responsivity data were reanalyzed by covariance techniques with conductance levels and skin temperature as multiple covariates, the drug effects were greatly reduced but did not disappear completely.

Relatively poor responders to stimulant drug therapy differed from good responders mainly in showing lower electrodermal responsivity both off and on drugs.

Significance to Biomedical Research and the Program of the Institute: Many investigators believe that the autonomic nervous system may be involved in this disorder. Therefore it is important to assess differences in autonomic functioning between MBD and normal children and to determine the autonomic effects of stimulant drugs which are effective in improving the behavioral problems.

Although this study does not confirm findings of lower arousal in MBD children (obtained by some, but by no means all, previous investigators), the data suggest that these children may "need" higher than normal arousal levels to perform effectively. The role of autonomic hyporesponsivity in the behavior deficits of the MBD children is not clear in view of the fact that the drugs which improve behavior do not increase, and may decrease, responsivity.

Proposed Course of Project: Continued data analysis, particularly with regard to the interrelationships between autonomic and behavioral variables. One paper has been submitted for publication, and another is in preparation.

Honors and Awards: None

Publications: Greenhill, L., Rieder, R. O., Wender, P. H., Buchsbaum, M. and Zahn, T. P. Lithium in the treatment of hyperactive children. Archives Gen. Psychiat. 28: 636-645, 1973.

Serial No. M-P-C(C) - #48
1. Laboratory of Psychology
2. Section of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Autonomic Functioning in MZ and DZ Twins.

Previous Serial Number: M-P-C-(C) - #17

Principal Investigator: Theodore P. Zahn

Other Investigators: Monte Buchsbaum

Cooperating Unit: None

Man Years:

Total:	.3
Professional:	.1
Other:	.2

Project Description:

Objectives: To determine the relative importance of genetic vs. environmental factors in various aspects of autonomic functioning. To determine the relationships of autonomic nervous system activity to personality, task performance and electrocortical functioning.

Methods Employed: Peripheral measures of autonomic functioning, namely skin resistance, heart rate, finger pulse volume, respiration, and skin temperature are recorded during two sessions which include rest periods, a series of mild tones, a reaction time task and mental arithmetic. The subjects are 30 MZ and 30 DZ twin pairs equally male and female. Some opposite sex DZ pairs have also been tested.

Intraclass correlation will be the primary method of analysis. It will be based on base levels, responsivity to non-demanding and demanding stimuli, variability and response specificity.

Major Findings: None, as yet.

Significance to Biomedical Research and the Program of the Institute: Aside from the inherent interest in the basic question of the heritability of autonomic indices and patterns, the data from this project will be used to help interpret the earlier findings from a similar study on schziophrenic MZ twins (M-P-C-(C)-#17).

Further Course of Project: Continued data analysis. Correlation of autonomic data with personality, behavioral and electrocortical data on the same subjects.

Honors and Awards: None

Publications: None

Serial No. M-P-D-(C)-30
1. Laboratory of Psychology
2. Section of Early Learning and Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Stimulus Conditions, Infant Behaviors, Caretaker-Child Interaction, and Social Learning in Diverse Child-Rearing Environments

Previous Serial Number: Same

Principal Investigator: Jacob L. Gewirtz, Ph.D.

Cooperating Units: Department of Psychology, Hebrew University of Jerusalem; Social Science Statistical Center, University of Maryland Baltimore County

Work on this project is temporarily suspended and is being held in abeyance.

Serial No. M-P-D-(C)-34

1. Laboratory of Psychology
2. Section on Early Learning and Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Contextual Determinants of Stimulus Power

Previous Serial Number: Same

Principal Investigator: Jacob L. Gewirtz, Ph.D.

Other Investigators: None

Cooperating Units: University of Maryland Baltimore County

Work on this project is temporarily suspended and is being held in abeyance.

Serial No. M-P-D-(C)-42
1. Laboratory of Psychology
2. Section on Early Learning and Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Evaluation of Concepts Employed for Early Learning and Development

Previous Serial Number: Same

Principal Investigator: Jacob L. Gewirtz, Ph.D.

Other Investigators: None

Cooperating Units: None

Work on this project is temporarily suspended and is being held in abeyance.

Serial No. M-P-D-(C)-43
1. Laboratory of Psychology
2. Section on Early Learning and Development
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mutual Learning Implications of Mother-Infant Interaction

Previous Serial Number: Same

Principal Investigator: Jacob L. Gewirtz, Ph.D.

Other Investigator: Elizabeth Boyd, Ph.D.

Man Years:

Total	4.00
Professional:	2.00
Others	2.00

Project Description:

Objectives: In past years, theoretical and empirical approaches to child behavioral development have been based for the most part on the one-sided assumption that parent (or other caregiver) behaviors are the main environmental influencers of child behaviors and development. An implicit corollary assumption has been that if the child's behaviors could at all influence those of his parent, that influence could play but a minor role in the child's socialization. In this vein, learning approaches have been concerned with the conditionability of selected infant behaviors by actual or simulated aspects of the social environment. However, during the past decade the issue of the direction of socialization effects has become increasingly focal for diverse approaches to early child development and to mother-infant interaction. Beginning in the late 1950s, our approach to this matter has been conceived in a learning framework, the central assumption of which has been that, at the very same time as the mother may be conditioning (reinforcing) some of her infant's behavior(s), her infant may be conditioning various of the mother's own behavior(s). This conception has provided a basis for embarking upon the present systematic, experimental analysis of the sequential features of parent-infant interchanges, directed to uncovering the mutual influence process inherent in mother-infant interaction. Our working assumption is that the infant's behaviors can influence his mother's behaviors in the same situations in which her behaviors can influence his. This focus represents the attempt, experimentally, to move on from our earlier work on the dependencies between parent-caretaker and infant behaviors in natural, life settings. That work was necessarily limited insofar as it was not feasible to intervene in ongoing mother-infant interchanges to demonstrate the causal relationships underlying these interchanges. In the present experimental program, certain features of

the interaction between mothers and their infants are manipulated, to highlight systematically some critical aspects of that influence process. However, occasional probes of mother-infant influence patterns are also made under typical family home conditions, to monitor the validity of our experimental observations for the naturally occurring interaction.

Methods Employed: In this conceptual frame, research designs have been formulated to answer experimentally two concurrent questions: 1) Can particular contingent infant behaviors (actual or simulated) function to modify (reinforce) aspects of selected mother behaviors? and 2) What is the effectiveness of selected contingent mother behaviors in modifying features of particular behaviors of her infant? The designs employed are paradigmatic for extensive study of mutual instrumental conditioning of mother and infant, providing considerable flexibility to study the effects of diverse responses of the child (e.g., head turns, leg kicks, smiles, vocalizations, laughter, reaching toward) presented contingent on various postural, facial, and/or verbal responses of the mother.

Subjects are full-term, healthy, normal infants, approximately three months of age at the start, and their mothers.

Extensive pilot observations indicated that it would be difficult to achieve the degree of articulation between the responses of the infant and of the mother required to condition the behaviors of both simultaneously. We decided therefore to emphasize initially the conditioning of the mother's behaviors by the infant's and, afterwards, to attempt the mutual conditioning of mother and infant behaviors. Because the mother's attention span was in the experimental setting much longer than her infant's, it was also decided in this initial research phase to simulate the infant's headturning response that, under the instructions given, cued the mother's verbal response. Hence, we first concentrated on the impact of a simulate set of infant vocalizations on a two-stage response of the mother -- her verbal response followed immediatel by her smile -- in a context where the mother vocalizes one-or two-word statements whenever a sequence of lights has indicated that her infant had turned his head to look at her. Specifically, in the experimental setting the mother was seated in a lighted room facing a one-way mirror. She believed that her infant was seated directly on the other side of the mirror in an adjacent, darkened room, and that the light differential was required to provide her infant a clear view of her face so that he would attend to her without being readily distracted. This was required, she was told, to permit the investigation of whether infants like her own child could learn that their head turns lead to a consistent social consequence, a short verbal statement of their mothers (which learning would be denoted by a rise in their head turn rate). The mother was asked to provide this consistent social consequence by saying a one-or two-word phrase to her infant through a microphone when a sequence of lights was activated by his head turning. (In this connection, it was not possible for the mother's statements to be reinforced by the light sequences denoting her infant's simulated head turns as the latter occurred at a stable rate with a lengthy delay from the completion of any verbal phrase and the head-turn light cue. Further, the mother had been cautioned not to read too

much into the infant head-turn representations as the experimenters would, as a function of the infant's learning, routinely be changing the response components and amplitudes that qualified as a reinforceable response.) Ostensibly, a microphone in the infant's room was automatically activated (for 1 sec.) after the mother finished speaking, permitting her to listen to a sampling of her child's vocal behavior through an intercom positioned near her. In actuality, that intercom received output from a two-channel tape recorder, one channel of infant vocalizations and one channel of ambiant room noise indicative of the child's silence.

The results here reported were obtained from observations of the mother's differential usage of each of six words in a one- two-word phrase. On the basis of their similiarities of meaning and frequencies of baseline occurrence, these six words were combined into two classes. Each verbal class could be followed by a smile or not followed by a smile, thus constituting four two-phrase response classes: one verbal class with and without smile and the other verbal class with and without smile. During the initial conditioning phase, the verbal class followed by smile that was the lower in baseline incidence occasioned a simulated infant vocalization. The other three response classes were followed by simulated room noise. Following a reliable increase in frequency of the conditioned response class, the conditions were reversed. Recorded infant vocalizations were presented contingent on the other response class and room noise followed the previously conditioned response class. These reversal conditions were in effect until a reliable increase in the earlier nonconditioned verbal response followed by smiles was demonstrated together with a decrease in the frequency of the previously conditioned response class.

Major Findings: We here report the results from over 20 biweekly observations of two mother-infant pairs. These were the first studied under the above-described conditions, and are the ones completed to date. The patterns of change from condition to condition in each of these mothers separately were all statistically reliable. For the first mother, the verbal response category followed by smiling was reliably conditioned, reversed, and reconditioned. For the second mother, that interactive behavior was reliably conditioned, reversed, reconditioned, reversed again, reconditioned again, reversed again, and reconditioned again. These results attest to the efficacy of contingent vocal responses that these mothers assumed to be their own infant's in increasing in each mother the incidence of the two-stage social response of a vocalization followed by a smile. That is, in an interactive context like that which characterizes mother-infant interaction in life settings, mothers verbal and smile behaviors were conditioned by what they took to be responsive vocal behaviors of their own infants. Interview probes after sessions and the mothers' spontaneous remarks about their infants' performances indicated that these changes in response patterns occurred without the mothers being at all aware of the controlling contingencies operating or that their infants' behaviors were being simulated.

Scientific Significance to the Program of Mental Health Research: The reciprocal influence processed of mother (or other caregiver) and infant upon each other is an important key to understanding the products of mother-

infant interaction, as well as the adaptive learning of the child and of the parent caregiver. In this way, the principles identified bear directly upon possible aberrations in these learned outcomes, and hence upon the continuing mental health of parent and infant.

Proposed Course of Project: The experimental conditions outlined above are being implemented routinely. The first publications of the results of the project are in preparation. It is planned to replicate these results with additional mother-infant pairs. The attempt will then be made to extend the results in a context where the mother's two-phased verbal-smile response is cued by the infant's vocalization. At the same time, the effect of the infant's simulated head turning on the mother's verbal response and smiling will be assessed. Given reliable demonstration of the efficacy of these two simulated infant behaviors in controlling mothers' social, interactive behavior, the infant will be brought into the experimental setting. The mother will then be responding contingently to affect the infant's actual behavior of head turning (or vocalization), and the mother's verbal behavior-smile response or some other expressive behavior will be maintained differentially by the simulated infant behavior of vocalization (or head turning). The next step will be to program an infant-mother interchange paradigm such that a response of the mother (e.g., a facial expression, smile) will be maintained by the actual response (e.g., a vocalization) of her infant, for which this response of the mother serves as the cue. In order to train the infant to respond discriminatively to the mother's expressive facial response (like a smile), the mother would be cued to respond to her infant only after it had been observed that she had smiled. In this way, the infant's discriminative vocalization would function to maintain (reinforce) his mother's smile behavior, which behavior in turn would serve to cue her infant's vocalization. Hence, a sophisticated discrimination would be learned by the infant and be applied differentially to maintain, indeed to control, some of his mother's expressive behaviors. This procedure would constitute a step in a functional analysis of some salient elements of the actual process of interchange between mother and infant. In these ways, the research program would be moving ever closer to providing leverage upon the influence, at one and the same time, that the behavior of one interactor has on the behavior of the other, very much as is assumed to occur in the life situation.

Honors and Awards: None

Publications: None

Serial No. M-P-P-(C)-6
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Investigation of Some Formal Characteristics of Speech

Previous Serial Number: Same

Principal Investigator: Donald S. Boomer, Ph.D.

Other Investigators: Allen T. Dittmann, Ph.D.

Cooperating Units: None

Work on this project is temporarily suspended and is being held in abeyance.

Serial No. M-P-P-(C)-42
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Precocious Science Students in Psychiatric Treatment: A Longitudinal Study

Previous Serial Number: Same

Principal Investigator: Kenneth Burgdorf, Ph.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued.

Serial No. M-P-P-(C)-43
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Test and Performance Measures of Creativity in Science

Previous Serial Number: Same

Principal Investigator: Kenneth Burgdorf, Ph.D.

Other Investigators: None

Cooperating Units: None

This project has been discontinued.

Serial No. M-P-P-(C)-45
1. Laboratory of Psychology
2. Section on Personality
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development Factors in Conversational Behavior

Previous Serial Number: Same

Principal Investigator: Allen T. Dittmann, Ph.D.

Other Investigators: Donald S. Boomer, Ph.D.

Cooperating Units: Green Acres School, Rockville, Maryland

Man Years:

Total	1.75
Professional:	1.00
Other	.75

Project Description:

Objectives:

General: To study the development of adult-type interaction patterns of conversation from the dual standpoints of language comprehension and social feedback.

Specific: (1) To define conversation as a rule-governed social system, and to compare children's participation in that system with the established patterns found in adults. (2) In a related study, listening behaviors in a group situation, classroom teaching, are observed in three age groups. (3) Conversational responsiveness in adults is compared in an experimental situation, varying social parameters.

Methods Employed: (1) Systematic observations of children in a school setting, but not in focused teaching situations. The observational protocol includes: (1) speaker turns (when one speaker stops talking and another starts); (2) length of speech in each turn (determined by the number of rhythmically marked "chunks" of speech called Final Juncture Units, or FJUs); (3) the content of the talk dichotomized as immediate or remote (commentary about current activity vs. reference to activities removed in space or time); (4) the appearance of Listener Responses (LRs: head nods and brief vocalizations like "m-hm"); (5) whether an adult or a child is talking or responding; and (6) whether the situation is an instructional one or not. In addition a running account of

time is noted, along with the composition of the conversational group in terms of size, sex, and age. Observations are made in the 6th, 7th, and 8th grades of a private school where the method and purpose of the research has been explained to children and staff in person and to parents by letters.

(2) Time sampling techniques are used to study listening and non-listening behaviors of children in response to classroom teaching. The observational protocol includes 5 categories of listening behaviors and 10 categories of nonlistening behaviors. These categories are used as a checklist to be marked every 15 seconds for a 7-minute period. The sample consists of 8 children (four boys and four girls, randomly selected) in the 2nd, 4th, and 6th grades in each of two schools, each child observed 5 times inlanguage arts and 5 in science and math.

(3) Sixteen Normal Volunteers subjects in the Clinical Center of NIH, half men and half women, are studied in conversations under conditions where sex differences, combinations of same sex and other sex conversational partners, and new situation or familiar situation can be simultaneously compared in a factorial design. The subjects come to the experimental room in pairs, each subject conversing on one occasion with another of the same sex and on a second occassion with another of the other sex. Eash time the instructions are for one person to carry the main burden of the conversation for about 5 minutes, then the other for about 5 minutes. Recordings are made on video tape and audio tape for later analysis. The dependent variables are number of LRs per FJU and number of Content Responses (CRs) per FJU. As a side issue, the incidence of a certain type of smile that appears to serve as an LR along with nods and brief vocalizations is also studied.

Major Findings: (1) Preliminary findings only from the first study can be reported at this time. The original observational protocol was improved from the one last year to include more variables, and subsequently during 14 visits to the school 121 observations have been made on 37 occasions. Observations will continue until a balance of frequencies for grade, sex, adult-child participation, and immediate-removed topics is achieved. Even so, fairly large numbers can be reported at this time if restricted to totals: 1,369 speaker turns have been recorded, comprising 2,486 FJUs (these range between 5 and 20 words). The age difference appears already, along with a strong difference in responsiveness to children compared with to adults- - when adults are in the conversation it is highly likely to be instructional, even informally so, in a school situation, and LRs are far more frequent in instructional situations. One unexpected finding has emerged, a substantial sex difference both in length of speaker turns and in responsiveness, with boys being the more responsive, under all conditions and at all ages. Earlier results with children seen in the experimental room here at NIH, using video tape recordings to collect the data, a small but significant sex difference in the opposite direction had been seen. Full interpretation cannot be made until the present sample is more balanced.

(2) This study is in the data collection stage -- about three-fourths of the time samples have been made at this time.

(3) This study is also in the data collection stage. A running account of the LR smile has been made of those subjects whose records have already been completed (half the projected number). Only half the subjects use this type of smile to any appreciable extent, and those only for about a tenth of their LRs.

Scientific Significance to the Mental Health Research Program: Conversational behaviors lie at the interface between language and social responsiveness, and open a number of research and practical possibilities ranging from basic research about language learning to educational and diagnostic applications. From the standpoint of language learning, behavior of both speakers and listeners in conversations can tell us more about the development of mastery of both the encoding and decoding processes, as have been studied among adults in this Section for many years. It appears from early results that the acquisition and reliable use of language arrives at a stage of life far later than much of the past research in language learning has concentrated on. The traditional tie spoken of by educators between speaking, listening, and reading has had little substance to draw upon in the realm of listening, and so this research has obvious possibilities for application in education.

The development of the social aspect of conversation, that of exchange between two (or more) people, all of whom have specific functions in helping each other keep the process going, also has clear applicability. In developmental diagnosis there are few specific behaviors we are able to point to that might mark a child's progress toward maturity. The extent to which he participates in the rules of conversation can give us one set of specific behaviors to look at -- provided we can obtain age norms for comparison.

Proposed Course of Project: Partly to develop such norms for specified settings, and to explore the parameters of the findings already obtained.

Honors and Awards: None

Publications:

Dittmann, A. T. Style in conversation. Semiotica, 9: 241-251, 1973.

Dittmann, A. T. The body movement-speech rhythm relationship as a cue to speech encoding. In Weitz, S. (ed.): Nonverbal communication. New York: Oxford, 1974, pp. 169-181.

Dittmann, A. T. The role of body movement in communication. In Siegman, A.W., and Feldstein, S. (eds.): Nonverbal behavior and communication. Potomac, Md: Erlbaum, in press.

Serial No. M-P-A-16
1. Laboratory of Psychology
2. Section on Higher Thought Processes
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A Study of the Means-end Thought Processes in Human Subjects.

Previous Serial Number: Same

Principal Investigator: Edward A. Jerome

Other Investigators: None

Cooperating Units: Technical Development Section, NIMH, Computer Systems Laboratory, DCRT

Man Years:

Total:	3.00
Professional:	1.00
Other:	2.00

Objectives: It is the purpose of this project to investigate the mental activities that are responsible for resolving problematic situations that occur in the course of the individual's interaction with his environment. Problems often result from imperfect knowledge of the operational regularities of external systems, and resolution of the difficulty is achieved by competent inquiry into these regularities. In order to be effective, the inquiries must be guided by a set of cognitive principles collectively called heuristics processes. This project has the following objectives: to describe the distribution of heuristic capabilities in various cross-sections of the population, to discover means of facilitating the acquisition of effective heuristic principles, to find ways of improving people's ability to apply such principles, and to participate in the development of a normative theory of effective heuristic structures.

Methods Employed: Standard tests of mental ability are administered for comparison with performances on a variety of computer-controlled system analysis problems developed for this project. A set of 14 related classes of problems called HEPP has been described in previous reports. Three sets of new problems were developed recently for the new SEL 810B real-time computer configuration. The new programs monitor improved procedures for the selection, reception, and rule identification paradigms of experiments on concept formation. All of these programs implement special controls that were impossible (prohibitively expensive and/or cumbersome) without the support of a high

speed process-control computer.

The programs that control these procedures have now been organized into a system capable of directing four stages in the execution of psychological experiments: (1) the application of the general design, e.g., randomly or systematically assigning subjects and conditions; (2) giving orientation instructions and testing for comprehension; (3) executing the procedures assigned in (1) and recording the run-time results; (4) collating the data from the assigned sessions and formatting it for input into NIH-DCRT statistical programs. If stage (4) can be interfaced with the central computer, this system could free the investigator to devote his time to planning and interpreting experiments providing he could secure programming support. This work has, however, been seriously impeded by reductions in personnel.

The methods used in these studies consist in analyzing the responses of patients as they conduct an inquiry into the behavior of a system by interacting with a computer program through a push-button interface. The purpose of their inquiry is to resolve uncertainties about classification procedures used by the program. The uncertainties derive from one of two sources: (1) a question about which subset of a known set of properties is being used by the program in a classification rule, the form of which is known to the patient; (2) a question about which of a known set of rules is being used in conjunction with a known set of properties to determine a classification procedures. The information that the problem solver can elicit is of two kinds: one implies that certain properties (rules) are relevant (possible), the other implies that certain properties (rules) are irrelevant (impossible). Sufficient information to determine a solution usually requires coordinate use of both types of information together with an understanding of the full range of possibilities. The purpose of this research is, of course, to assess the abilities of individuals assigned to various diagnostic, social, educational and age categories to conduct competent inquiry and to use effectively the implications elicited by the inquiry.

Major Findings: In experiments of the general form used in this section, it is usually found that various logical operations governing rules of classification generate problems that differ in apparent difficulty--measured by the number of inquiries made by the patient. This operator-effect has been explained by two theories that are usually considered to be alternatives. The results of an experiment now being reported by the section indicate that while one of these hypotheses, an information-theoretic analysis, explains much of the observed difference, there is also a residual operator-effect that may be explained by the other.

A set of analytic indices has been developed by the section that make it possible to at least partially separate the effects of the factors proposed by these two theories; it is this analytic device that enables us to investigate the possibility that the two theories are complementary rather than competitive.

The conclusion derived from these considerations is that although mathematical properties of the problem spaces involved (objective complexity) determine the difficulty tasks to a large extent, there is also a more uniquely cognitive operator-effect that may require explanation in terms of a hierarchical theory of concept, or some hypothesis like it.

A priori analysis of the patient's task suggests that it has at least two components: (1) the accessing of data that are needed to support a conclusion and (2) the processing or interpreting of available data in order to identify the conclusion supported. These two tasks may be performed sequentially or alternately; i.e., data may be accessed with little or no effort to interpret it until some amount judged adequate has been stored, or an effort may be made to interpret each item of information as it is accessed--of course, various compromises between these extremes may be adopted.

The results of the section's experiments indicate that the sequential approach is characteristic of the novice; it creates a need for considerable redundancy and is associated with inefficient inquiry. With experience most people develop the "elicit-interpret" approach, and with it comes a much more sagacious sequencing of inquiries and an impressive reduction in the need for redundancy.

Significance to Biomedical Research and the Program of the Institute: The individual's ability to cope with the unexpected, unfamiliar and enigmatic situations that arise hourly in the course of normal existence is probably the most important determiner of his mental health and behavioral stability. The section's efforts are directed toward identifying defects in the cognitive processes responsible for successful coping and to search for methods of repairing them. To this end it constructs, organizes and tests theories of means-end thinking and develops methods of detecting, assessing, and correcting heuristics deficits.

Proposed Course of Project: For the coming year the section has two goals: (1) to complete an experiment on rule identification that is now in progress and (2) to reactivate a project on computer stimulation of theories of concept formation.

Honors and Awards: None

Publications: None

Serial No. M-P-B-2
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Analysis of the relationship between problem-solving behavior and certain cortical and subcortical structures in the sub-human primate brain.

Previous Serial No.: Same

Principal Investigators: H. Enger Rosvold, Patricia S. Goldman

Other Investigators: Charles Popper (Research Associate, Laboratory of Clinical Science)
Timothy Havens (Grant Foundation Fellow, Laboratory of Clinical Science)

Cooperating Units: Laboratory of Clinical Science

Man Years:
Total: 3
Professional: 4/5
Other: 2 1/5

Project Description:

Objectives: To study the neural mechanisms underlying cognitive behavior.

Methods: (1) Assessment of the behavioral deficits resulting from selective lesions of cortical and subcortical areas, particularly in the frontal lobe of the rhesus monkey. The cortical lesions are made by aspiration; the subcortical lesions by passing radiofrequency current through sterotactically placed electrodes. Behavioral tests include the classical delayed-response problems, object discrimination reversal and visual pattern discriminations. (2) To determine whether cortical lesions result in disturbance of biogenic amine metabolism in subcortical structures, monkeys were given unilateral dorsolateral lesions (n=8), unilateral orbital lesions (n=8), or employed as unoperated controls (n=8). Approximately 18 days after surgery, the monkeys were sacrificed and the following structures were immediately dissected: caudate nucleus, septal nucleus, locus coeruleus, hypothalamus, anterior thalamus, hippocampus, cingulate cortex. These structures have been analyzed by standard fluorometric methods for levels and turnover of norepinephrine, dopamine and serotonin. (3) Bilateral cannulas are implanted directly over specified regions of the frontal cortex in monkeys trained to perform delayed alternation and visual discrimination problems. The effect on behavior of injecting five putative

neurotransmitters (norepinephrine, dopamine, serotonin, acetylcholine and histamine) at various doses will be examined. If specific effects are obtained for particular agents, these will be candidates for more analytic studies involving the action of antagonists and the effects of drugs which potentiate or inhibit neural transmission.

Major Findings: (1) The description of behavioral deficits following selective lesions of the frontal cortex has been worked out in a long series of experiments spanning the history of this section. Recently completed work, described in last years annual report, has confirmed the major findings and has provided further specification both with respect to the lesions and to the nature of the behavioral impairments. Present research is directed toward working out the behavioral functions of the subcortical structures that entertain preferential anatomical connections with prefrontal cortical areas. One of these subcortical structures is the dorsomedial nucleus of the thalamus. It has been a long-standing puzzle in neuropsychology that lesions of this nucleus did not result in "frontal" deficits despite the intimacy of its anatomical connections with the frontal cortex. Nevertheless, since the anatomical relationships are so compelling we undertook to investigate this problem again, this time with positive results. So far, twenty-two monkeys (nine infants and fourteen young adults) have been given lesions intended to damage the entire nucleus, various divisions within it, or thalamic structures adjacent to it. The results clearly indicate that (a) damage to the dorsomedial nucleus reliably produces impairments on the more demanding "frontal-lobe" problems; (b) lesions of the dorsomedial nucleus result in selective deficits in infants as well as adults; (c) the deficits depend on substantial but not necessarily complete destruction of the nucleus; and (d) damage to the division of the nucleus which projects to the orbital prefrontal cortex (magnocellular) appears to be more essential than damage to the division which projects to the dorsolateral cortex (parvocellular). It is anticipated that this project will soon be completed. Another of these subcortical structures having close anatomical relationships with the prefrontal cortex, is the hypothalamus. While many studies have demonstrated similarities between the frontal cortex and the hypothalamus in the emotional and motivational functions which they subserve, none has considered whether or not there are also similarities in cognitive functions. Our recent work, still in progress, has shown that large lesions involving different divisions of the hypothalamus result in a pattern of deficits on cognitive problems which are characteristic of damage to the orbital frontal cortex. Smaller, selectively placed lesions, though resulting in impairments, did not yield a pattern of deficits unequivocally characteristic of one or the other of the frontal-lobe systems. (2) This study is addressed to four specific questions: (a) Do cortical lesions produce metabolic disturbances in subcortical structures? (b) Are the disturbances confined to the damaged hemisphere or are they bilateral? (c) Is the degree of disturbance related to the anatomy, i.e., are monosynaptic pathways more severely affected than polysynaptic connections, etc.? and (d) Can the major functional subdivisions (dorsolateral vs. orbital) of the prefrontal cortex be differentiated in terms of neurochemical pathways? The experiment is in the data-analysis stage and should be completed within the year. (3) Five animals have completed preoperative training and have

had cannulas implanted bilaterally over (a) the principal sulcus, and (b) the orbital gyri. Three of these animals have also completed an initial post-operative testing sequence to establish whether the implantation of cannulas per se would produce even slight impairments. The results show that the surgical procedure is without effect on performance and the monkeys are now ready for pharmacological intervention. Although the psychoactive effect of many drugs is plainly evident, it is rarely clear where in the brain such drugs may be acting to alter behavior. Approaches to the problem of local action that involve either systemic or intraventricular administration of drugs are inadequate in that regional specificity cannot be claimed. By injecting radio-opaque dyes through cannulas (implanted as above) in untrained monkeys, we have determined from x-rays that the distribution of compounds injected through the cannulas is restricted to the target region - thereby establishing the feasibility of local intracerebral injections. This approach may shed light on the neurochemical pathways involved in frontal-lobe mediation of behavior and at the same time provide a means of elucidating how drugs administered systemically can affect behavior by acting on specific regions of the brain.

Scientific Significance to Mental Health: The results of this project have revealed that the prefrontal cortex in monkeys is functionally heterogeneous with dorsal and ventral areas participating in problem-solving behavior in two quite different ways; even within these areas there is evidence of further specialization of function. Furthermore, each of these frontal areas appears to be but one component of a larger cortical-subcortical system. Specification of the types of cortical contributions to problem-solving, and delineation of the two-cortical-subcortical systems, in terms of anatomy, neurochemistry, and behavior should help to resolve the long-standing mysteries surrounding the functions and perhaps also the mechanisms of this large and evolutionarily significant region of the primate brain.

Honors and Awards: Mr. Leon Dorsey received a cash award for Superior Work Performance.

Publications:

Marburg, D. L. The effect on reaction to painful stimuli of lesions in the centromedian nucleus in the thalamus of the monkey. Intern. J. Neurosci. 5: 153-158, 1973.

Marburg, D. L. The effect of lesions in the centromedian nucleus of the thalamus of the monkey's performance in delayed alternation and object reversal tasks. Intern. J. Neurosci. 5: 207-214, 1973.

In Press:

Bowden, D. M. and McKinney, W. T. Effects of selective frontal lobe lesions on response to separation in adolescent rhesus monkeys. Brain Res.

Bowden, D. M., Galkin, Thelma W., and Rosvold, H. E. Plasticity of the drinking system as defined by electrical stimulation of the brain. Physiol. and Behav.

Serial No. M-P-B-5
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neural mechanisms in vision.

Previous Serial No.: Same

Principal Investigator: Mortimer Mishkin

Other Investigators: Charlene D. Jarvis (NIMH Staff Fellow)
Jean Delacour (NIMH Guest Worker from College de France, Paris)

Cooperating Units: Charles Gross, Princeton University, Princeton, New Jersey
Helen Mahut, Northeastern University, Boston, Massachusetts
Frederick J. Manning, WRAIR, Washington, D. C.

Man Years:
Total: 5 4/5
Professional: 2 1/5
Other: 3 3/5

Project Description:

Project: Behavioral, anatomical, and electrophysiological analysis of neural mechanisms in vision.

Objectives: To define the role in vision of neural structures outside the primary visual projection system.

Methods: (1) Monkeys are prepared at NIMH with various lesions to the visual system, and they are then studied electrophysiologically at Princeton University by Gross and his colleagues to determine whether or not the receptive field properties of inferotemporal units have been altered in a manner predicted by our theory of cortico-cortical transmission. (2) Monkeys are tested after various inferotemporal and limbic lesions for visual memory (delayed matching-from-sample) and visual associative learning (discrimination reversal) in an attempt to determine whether these two mnemonic functions can be dissociated, and selectively related to the anterior inferotemporal cortex and to the temporal pole and amygdala, respectively. (3) Monkeys are prepared with lesions of the temporal pole and amygdala in one hemisphere, the inferotemporal cortex in the other, and either partial or complete forebrain commissurotomies; they are then tested on the behavioral tasks described in (2) in order to discover whether either visual or associative memory depends on the anatomical

pathway connecting the inferotemporal and temporal limbic regions. (4) Unit activity in the inferotemporal and polar regions is recorded while a monkey performs a visual discrimination reversal to determine whether inferotemporal units might be sensitive to the sensory and not the affective quality (reward value) of a stimulus, and conversely, whether polar units might be sensitive to the affective and not the sensory quality.

Major Findings: (1) In accord with predictions, left striate cortex ablation restricts the receptive zones of inferotemporal units in both hemispheres to the left visual field, while forebrain commissurotomy limits the units' receptive zones to the visual field contralateral to the hemisphere being recorded from. The latter effect appears to be due primarily to transection of the splenium of the corpus callosum: transection of the anterior commissure by itself has no detectable consequence. (2) Preliminary data suggest that both the anterior inferotemporal cortex and the temporal pole and amygdala contribute to both types of mnemonic mechanisms (visual and associative) though there may be relative differences between them; by contrast, the hippocampus appears to contribute to neither type of memory. (3) Preliminary results indicate that cutting the anterior commissure alone in a "crossed-lesion" preparation will reproduce the effects on reversal learning of a bilateral removal of the temporal pole and amygdala. (4) Unit recording in a behaving animal is now underway.

Scientific Significance to Mental Health Research: Vision is perhaps the best understood of the sensory modalities in relation to the functioning of the nervous system. Yet a major problem remains unsolved in vision as it does in all sensory modalities: Delineation of the events which intervene between stimulus reception at the cortex and the observed response. The demonstration that damage to the inferior convexity of the temporal lobes in monkeys produces impairment in visually-guided behavior has opened up the hitherto inaccessible area of the intracerebral processes in vision, i.e., neural activity related to vision but beyond the level of the striate cortex. Unravelling these mechanisms in vision should aid greatly in the solution of a general problem for psychology, viz., accounting for the intervening neural processes (thought and its breakdown) in normal and abnormal behavior.

Proposed Course of Project: (1) Monkeys with forebrain commissurotomies that leave intact only the splenium or only the anterior commissure are being added to the electrophysiological study in order to determine the precise pathway responsible for visual activation of inferotemporal neurons. (2) The TPA ablation will be divided into two lesions (TP vs A) to test whether the two types of memory impairment can be dissociated. (3) Controls for the "crossed-lesion" preparations will be added (e.g., TPA x striate + ant. comm., and TPA x IT + corp. call.) to test the selectivity of the lesion-deficit relationship. (4) This study will be pursued through initial data gathering, at which time an evaluation will be made re continuing vs modifying it.

Honors and Awards: None

Publications:

Iversen, Susan D. and Mishkin, M. Comparison of the effects of superior temporal and inferior prefrontal lesions on auditory and non-auditory tasks in rhesus monkeys. Brain Res. 55: 355-367, 1973.

Niki, H. Prefrontal unit activity during delayed alternation in the monkey: I. Relation to direction of response. Brain Res. 68: 185-196, 1974.

In Press:

Jarvis, Charlene D. Visual discrimination and spatial localization deficits after lesions of the tectofugal pathway in pigeons. Brain, Behav. and Evol.

Robinson, D. L. and Jarvis, Charlene D. Superior colliculus neurons studied during head and eye movements of the behaving monkey. J. Neurophysiol.

Rocha-Miranda, C. E., Bender, D. B., Gross, C. G., and Mishkin, M. Afferent pathways underlying the visual response properties of inferotemporal neurons. J. Neurophysiol.

Serial No. M-P-B-7
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Histological analysis of cerebral lesions and intracerebral connections in primates.

Previous Serial Number: Same

Principal Investigator: H. E. Rosvold, M. Mishkin, Patricia S. Goldman

Other Investigators: T. N. Johnson (Consultant Part-time)

Cooperating Units: None

Man Years:
Total: 2 4/5
Professional: 3/5
Other: 2 1/5

Project Description:

Project: Verification of lesions in chronic experimental animals, and analysis of intracerebral connections in acute preparations.

Objectives: The objective of the first part of this project is to delineate the loci of the lesions which produce specific behavioral effects, and of the second, to determine whether or not loci yielding similar effects are anatomically interconnected.

Methods: When the behavioral testing of chronic experimental animals is completed, their brains are embedded in either celloidin (large specimens) or paraffin (smaller specimens), sectioned, stained for cells and fibers, examined microscopically, and the lesions reconstructed. In the second part of the project, animals are prepared with selective lesions purely for anatomical investigation. The animals are sacrificed within a few days after surgery and their brains examined microscopically to determine the locus, extent and course of degenerating fibers. Special staining procedures and fiber-size determinations are used to study stage of myelinization.

Major Findings: Specimens completely processed and examined include 118 monkey brains. This material has been examined for the purpose of verifying the loci of the cortical and subcortical lesions in animals used for behavioral studies.

In previous studies it had been demonstrated that there is a topographic projection of fibers from various parts of the prefrontal cortex to the head of the caudate nucleus and putamen. Furthermore, this topographic arrangement is maintained in the efferent projections of the caudate to the globus pallidus, fibers from the dorsolateral caudate taking a lateral position in the pallidum, and fibers from the ventrolateral caudate taking a medial position in the pallidum. Current work suggests that, in turn, the efferents from the lateral pallidum and from the medial pallidum are dissociable, each taking separate trajectories into the thalamus and subthalamus. However, while the projection of the lateral pallidum is rather selectively to the subthalamic nucleus, the projection of the medial thalamus seems to be more disseminated through the thalamus and subthalamus. The reliability of these findings is being ascertained in additional preparations.

Scientific Significance to Mental Health Research: Sufficient evidence has been accumulated in behavioral studies with brain-damaged animals to suggest that widely separated cell aggregates in cortex and subcortex may be combined into a complex system; and it is the functioning of such neural systems, rather than the functioning of the particular elements of which they are composed, that seems most directly relevant to an understanding of the organization and disorganization of behavior. The significance of this project lies in the fact that it can provide detailed information on the composition of these neural systems; systems which through electric and chemical manipulation have been related to mental health and disease.

Proposed Course of Project: The studies involving localization of function, anatomical substrate of the frontal systems, and anatomical substrate of the visual systems will continue.

Honors and Awards: None

Publications: None

Serial No. M-P-B-16
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cerebral mechanisms underlying functional plasticity in the developing organism.

Previous Serial No.: Same

Principal Investigator: Patricia S. Goldman

Other Investigators: H. Enger Rosvold

Cooperating Units: Edward Taub and Epp Miller, IBR, Silver Spring, MD

Man Years:
Total: 2 4/5
Professional: 3/5
Other: 2 1/5

Project Description:

Objectives: The purpose of this research is twofold: to study the relationship between structure and function in normal development and to determine the neural mechanisms involved in recovery of function following brain injuries, particularly those sustained in infancy.

Methods: (1) Monkeys given prefrontal cortical lesions at 50 days of age and unoperated controls were tested on a variety of behavioral measures after they had reached two years of age; (2) Monkeys were given prefrontal cortical lesions at 50 days of age and tested initially at 2 1/2 months of age; (3) Monkeys that had been given prefrontal lesions in infancy and had demonstrated recovery of function at two years of age were subjected to additional removal of other cortical areas at the two-year stage of development. Behavioral status was then reassessed; and (4) Nine monkeys were given prefrontal cortical lesions in utero at the end of the second trimester of pregnancy. Viable offspring will be assessed behaviorally and anatomically.

Results: (1) Previous research had shown that monkeys given dorsolateral lesions in infancy could perform delayed-response as well as unoperated controls when tested at 1 year of age but the same monkeys began to exhibit deficits when they were retested at two years of age. In the present study, monkeys with dorsolateral lesions were initially tested at the two-year stage of development. Again, the operated monkeys were impaired, even though their lesions were incurred in infancy. These findings extend the evidence for the late development of the dorsolateral cortex and, in addition, impose

restrictions on the degree of plasticity that can be claimed for the immature nervous system. (2) Although previous studies indicated that the functions of the orbital cortex were well developed by one year of age, they did not provide evidence on how early within the first year of life such functions were present. Our new results show that functional loss can be detected as early as 2 1/2 months of age, but only in males. The evidence shows that while orbital lesions produce deficits in male monkeys as early as 2 1/2 months of age, similar deficits cannot be detected in females until 15 months of age. These results suggest that the orbital cortex develops earlier in males than in females. Further, at least in males, there is a time lag of nearly two years between the functional development of the orbital and the dorsolateral prefrontal cortex. Differences of this magnitude in (a) the time-table for maturation of two different regions in males and in (b) the time-table for development of one of these regions in males and females provide an unprecedented opportunity for the analysis of structure-function relationships in development. (3) Monkeys raised from infancy without the orbital prefrontal cortex are impaired when initially tested at 2 1/2 or 12 months of age (males) or at 15 months of age (females). However, in spite of their initial deficits (which establish the functional maturity of the orbital cortex at early ages) these monkeys exhibit considerable "recovery" of function when they are retested at two years of age. Evidence obtained last year indicates that the recovery phenomenon depends on the integrity of the dorsolateral cortex and on the opportunity for experience during development. If the dorsolateral cortex is removed along with the orbital cortex in infancy or if the dorsolateral cortex is removed at two years of age, the recovery is abolished. If monkeys are raised from infancy without orbital prefrontal cortex and are initially tested at two years of age without benefit of prior experience, recovery is also abolished. Our evidence suggests that as long as orbital damage occurs in infancy the dorsolateral cortex develops normally and experiential factors aid in this development. The "recovery" exhibited by monkeys with orbital lesions sustained in infancy is thus viewed as the emergence with age of new capacities added by the normal development of intact structures rather than the expression of unusualy plasticity. (4) Two out of the nine fetuses survived to term and were delivered by cesarean section. Of these, one died of a respiratory infection after several weeks. The surviving monkey, a healthy animal with no apparent sensory or motor defects, reached his first birthday in February, 1974. Testing of this animal commenced in February and will continue throughout this year. The monkey could perform delayed-response as well as other monkeys operated postnatally. No other findings are available at this time.

Scientific Significance: By understanding mechanisms for recovery of function following neonatal injury, we may be in a better position to correct errors of normal development as well as approach the problem of recovery in the adult-brain injured.

Proposed Course of Research: (1) To study the effects of gonadal hormones at critical early periods on the development of the orbital cortex in males and females and (2) to investigate anatomical, physiological and biochemical correlates of behavioral development in the monkey.

Honors and Awards: None

Publications:

Miller, E. A., Goldman, P. S., and Rosvold, H. E.: Delayed recovery of function following orbital prefrontal lesions in infant monkeys. Science 182: 304-306, 1973.

In Press:

Goldman, Patricia S. Recovery of function after CNS lesions in infant monkeys. In Functional Recovery After Lesions of the Nervous System (E. Eidelberg and D. G. Stein, Eds.). Neurosciences Res. Prog. Bull.

Goldman, Patricia S. An alternative to developmental plasticity: heterology of CNS structures in infants and adults. In CNS Plasticity and Recovery of Function (D. G. Stein, J. Rosen, and N. Butters, Eds.) Academic Press, New York.

Goldman, Patricia S. Age, sex, and experience as related to the neural basis of cognitive development. In Brain Mechanisms in Mental Retardation (N. Buchwald, Ed.) Academic Press, New York.

Serial No. M-P-B-17
1. Laboratory of Psychology
2. Section on Neuropsychology
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neuroanatomical basis of function development and investigations of structural correlates of behavioral recovery following early brain damage.

Principal Investigators: Patricia S. Goldman, H. E. Rosvold

Other Investigators: T. N. Johnson (Consultant, Part-time)

Cooperating Units: None

Man Years:
Total: 1 1/5
Professional: 3/5
Other: 3/5

Project Description:

Objectives: (1) To describe the development of cortical-subcortical connections in normal ontogeny; (2) To search for evidence of new anatomical pathways resulting from early brain injury that would be capable of mediating behavioral recovery.

Methods: (1) Monkeys are given unilateral orbital or dorsolateral prefrontal lesions at 2 months, 6 months and 24 months of age. Five or fourteen days following surgery, the monkeys are sacrificed and their brains processed by the technique of silver impregnation, as well as by conventional methods of cell staining. Frontal sections through these brains are examined microscopically for evidence of anterograde and retrograde degeneration. (2) Monkeys that received bilateral orbital prefrontal lesions as infants and have demonstrated recovery of function at two years of age will be given unilateral lesions in regions of the brain hypothesized to be involved in mediating the recovery. These areas include the dorsolateral prefrontal cortex, the premotor cortex, the medial frontal cortex, the anterior temporal lobe and the amygdala. Controls are monkeys that receive unilateral lesions of the above areas at the two-year stage of development, without having had bilateral orbital surgery in infancy. Some of the preparations in (1) can serve as controls in (2). Following the unilateral surgery at two-years, the monkeys are sacrificed and their brains processed as in (1).

Major Findings: Degenerating fibers have been observed in the caudate nucleus and the dorsomedial nucleus of the thalamies following dorsolateral and orbital lesions at both 6 and 24 months of age. Following lesions at 2 months of age,

however, fiber degeneration was observed only in the thalamus (and in other structures, e.g., internal capsule) but not in the caudate. The findings were the same whether the survival time was 5 or 14 days. The results suggest that cortico-striatal connections are either immature or not present in the 50-day old rhesus monkey.

Scientific Significance to Mental Health: The brain is the organ of normal and abnormal behavior. Neuroanatomical studies together with behavioral, electrophysiological, and neurochemical approaches that shed light on how the brain develops provide a basis for understanding errors of development. Developmental anatomical studies may increase our understanding of the limits of central nervous system plasticity not only in relation to recovery from traumatic injury but with respect to experiential and hormonal factors that may speed or retard development.

Proposed Course of Research: Confirmation of present results with other anatomical techniques, e.g., autoradiography, horseradish peroxidase.

Honors and Awards: None

Publications: None

Serial No. M-P-L-5
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Individual Differences in Normal Perceptual Processes

Previous Serial Number: Same

Principal Investigator: V. R. Carlson

Other Investigators: Irwin Feinberg, Eugene Tassone, John Knight

Cooperating Units: VA Hospital, San Francisco, DCRT

Man Years:

Total:	2.0
Professional:	1.0
Others:	1.0

Project Description:

Objectives: General: (1) To obtain normal control data for standardizing procedures and apparatus in the measurement of perceptual variables, and to obtain comparison data for evaluating the effects of abnormal and other special conditions. (2) To develop a general specification of perceptual response in terms of the parameters of the immediate stimulus-situation, past experience, generalized perceptual-cognitive attitudes, and the subject's motivational reaction to the situation.

Postulation of a "biological clock" is fundamental to many physiological and psychological processes. Normally, human subjects can reproduce presented time-intervals with great accuracy. In verbal estimations of time, longer intervals are typically underestimated and shorter intervals are overestimated, resulting in judged absolute accuracy for an intermediate interval of physical clock time. Both the accuracy of reproduction and the point of absolute accuracy of estimation vary on different occasions. But they do not vary consistently with each other, nor do they vary systematically with appropriate changes in physiological or psychological state.

The specific purpose of the present research was to develop a model of the "internal clock" in terms of which the above and other aspects of the variability of human time judgment may be understood and related to variations in other biological processes.

Methods Employed: A theoretical model was specified in which the rate of the subjective passage of time is reflected in the slope of the response x time function and systematic errors of response are reflected in the intercept of the function. Based on this model a computer is programmed to simulate the time judgment behavior of human subjects. The simulated results are then compared with the actual time judgments of subjects obtained under various conditions. Estimation, production, and reproduction of time intervals have been obtained under conditions of reduced arousal, changing body temperature, and extended repeated testing.

Major Findings: Principally, the simulated results show: (1) A reciprocal relationship between the estimation and production of time intervals. (2) Random variation of the reproduction of time intervals around objective accuracy. (3) Response variance as a linearly increasing function of the square of the time interval being judged. (4) Determination of the estimation point of absolute accuracy by the level of systematic response error and the response variance function. Data from actual subjects correspondingly exhibit these same characteristics. In addition, as predicted, the slopes, but not the intercepts, of the response x time functions varied systematically with changing level of arousal.

Scientific Significance to Mental Health Research Program: Disorientation in time sense is often an accompaniment of abnormal psychological states. Reliable assessment of time judgment ability, however, has in the past been singularly unsatisfactory, logically comparable methods of measurement yielding inconsistent results. The present model appears to provide mutually compatible results for different methods of measurement and indicates that subjective time rate is slowed under conditions of reduced mental functioning.

Proposed Course of Project: Applicability of the model to the assessment of time judgment in altered psychological states is continuing by testing subjects under conditions of THC intoxication.

Honors and Awards: Election to Psychonomic Society, 1973.

Publications:

Carlson, V.R., and Knight, J.: SCV--A general subroutine for the selection of combinations of variables. Behav. Res. Meth. & Instru. 5(5): 425-427, 1973.

Tassone, E.P., Feinberg, I., and Carlson, V.R.: A computer program for the analysis of scored sleep EEG data. Psychophys. 10(6): 612-613, 1973.

Serial No. M-P-L-7
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Perceptual Adaptation

Previous Serial Number: Same

Principal Investigator: V. R. Carlson

Other Investigators: Eugene Tassone, Harry Blum, Francine Frome

Cooperating Units: DCRT, University of Maryland

Man Years

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives: General: To investigate the characteristics of perceptual adaptation, both when the adaptation occurs in a single limited period of persistent stimulation and when it is cumulative in a series of stimulations over relatively long periods of time.

The occurrence of visual adaptation to stimulus motion, form, and orientation suggests the operation of specialized receptive units in man of the kind which have been identified in cat and monkey visual cortex. Such adaptation is also basically similar in some respects to a conditioning or learning process. We have previously found that adaptation established to stimulus motion did not dissipate over a period of neutral stimulation and in that respect resembled a conditioning process more than a strictly sensory process. Specialized receptive units may be differentially sensitive to combinations of motion and color or form, however, and it is necessary to investigate the dissipation of adaptation to such combinations of stimuli.

Methods Employed: Subjects are simultaneously adapted to two different combinations of stimulus motion and color or form. Then the persistence of adaptation to both combinations is measured after increasing periods of exposure to neutral stimulation.

Major Findings: Apparatus suitable for presenting the necessary combinations of stimuli is under construction, and data collection has not as yet begun.

Scientific Significance to Mental Health Research Program: Specialized receptive neurons sensitive to particular combinations of stimuli probably exist in the human brain and are necessary for the perception of color, motion, form, and orientation. But even such specialized units do not seem to be sufficient to account for the kinds of perceptual adaptation that occur. An understanding of how perception develops will most probably have to be concerned with conditioning, learning, and memory processes as well as with basic sensory processes.

Proposed Course of Project: If the results indicate that a conditioning process is involved, it will be pertinent to investigate the variety of combinations of stimuli to which adaptation can occur. Sensory adaptation implies the effectiveness of a limited set of combinations. Conditioning implies a greater arbitrariness as to the specific nature of the stimulation which can be effective.

Honors and Awards:

Consulting Editor, Journal of Experimental Psychology, 1967-

Associate Editor, Perception & Psychophysics, 1971-

Publications:

Frome, F.: A psychophysical study of shape alignment. University of Maryland, Tech. Rep. TR-198 GJ-32258X, August, 1972.

Serial No. M-P-L-9
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Discriminative and Conceptual Behavior in Preschool Children

Previous Serial Number: Same

Principal Investigator: Albert J. Caron

Other Investigators: None

Cooperating Units: Private and Cooperative Nursery Schools in Montgomery County and the District of Columbia

Man Years: None

This project was temporarily suspended and is being held in abeyance.

Honors and Awards: None

Publications: None

Serial No. M-P-L-10
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Discriminative and Conceptual Behavior in Infancy

Previous Serial Number: Same

Principal Investigator: Albert J. Caron

Other Investigators: Rose F. Caron

Cooperating Units: George Washington University

Man Years:

Total:	2.25
Professional:	1.50
Others:	.75

Project Description:

Objectives: To assess the perceptual-cognitive capacities of human infants during the first year of life and to investigate the antecedent environmental conditions promoting early perceptual and intellective development.

Study I: The general aim of an extensive project is to provide a fine-grained analysis of the effects of early visual experience on the development of discriminative competence in infancy. One specific objective is to examine the respective roles of reinforcement and responding in promoting shape discrimination in the 3 to 6 month period. In so doing, the research is intended to resolve competing theoretical views regarding the necessity and sufficiency of differential reinforcement and differential responding in early perceptual learning. A second purpose of the project is methodological: to adapt discriminative operant technology to the special condition of the young human infant and to refine existing procedures for assessing the perceptual capacities of babies.

Methods Employed: The study employs both discriminative assessment procedures and discriminative operant training procedures. The former make use of the habituation-dishabituation or familiarization-novelty principle, namely, that attentional responding to prolonged or repeated stimulation tends to decline, and then recovers to a novel stimulus or a perceived stimulus change. Recovery of attention, thus, can be inferred to reflect the ability to discriminate

familiarized from novel stimuli. The technique is being used to locate geometric shapes which are non-discriminable by the average four-month-old and hence which can be used for visual training of three-month-olds. A number of procedural choices are open to the investigator employing the discriminative operant training technique, depending on whether he wishes to use one response or two, and, if two, the same or different reinforcers and/or the same or different schedules of reinforcement. At present we are following the classical discriminative operant procedure, using one response (head-turning) plus two reinforcement schedules (variable interval vs. extinction). Thus, in the presence of one discriminative stimulus (e.g., a circle), a head turn after a brief variable interval produces music and visual effects (spinning mobiles, moving lights, animated color cartoons), whereas in the presence of a contrasting stimulus (e.g., an x) the same head turn yields no effects. Different groups of three-month-olds will be exposed over a one month period either to this basic paradigm or to variants of it which differ in terms of (a) the correlation of differential reinforcement with the discriminanda, (b) the necessity for instrumental responding (non-contingent reinforcement being provided in association with the positive stimulus), and (c) the number of discriminative cues exposed. Following training, all infants will be assessed via the habituation-dishabituation procedure for their ability to discriminate the shapes seen in training and related stimuli.

Major Findings: The preliminary assessment phase of the project, which has just been completed, has yielded a number of geometric patterns which appear to be non-discriminable at four months of age, e.g., circle vs. x, circle vs. triangle, and circle vs. square. The circle and x are currently being used as discriminanda in our initial discriminative operant training sessions with infants. Progress in the discriminative training phase of the study has been primarily methodological and procedural. Since there is almost no technological information available on infant discrimination training, we were forced to confront the multitude of psychological problems posed by the need to harness this technology to the peculiar condition of the young human infant. These included: (a) location of sufficiently potent reinforcers to maintain behavior over extended time periods, (b) selecting instrumental responses which require minimal effort, recur frequently, and are easily and safely coupled to electronic devices, (c) determining optimal physico-spatial arrangements between discriminanda, responses, and reinforcers so as not to exceed the limits of the infant's orienting capabilities, and (d) determining which reinforcement schedules could be tolerated by young infants. Thus far, we have found that (1) moving stimuli (whether two- or three-dimensional) are much more powerful reinforcers than static stimuli, (2) visual feedback is more potent than auditory feedback, (3) music is superior to human conversation, (4) moving colored shapes are more powerful than moving black and white shapes, (5) trial to trial variation of any of the above, as well as session to session changes in their general characteristics, intensifies their reinforcing properties, (6) head-turning and leg-kicking can be brought under contingency control at three months of age, and (7) at three months infants can tolerate repeated extinction segments, as long as their duration does not exceed 30 sec., and can also perform under intermittent schedules (both FR and VI).

Study II: The purpose of a second study is to determine whether the three- to four-month-old infant has achieved perceptual shape constancy, i.e., whether objects retain their shape despite shifts in perspective.

Methods Employed: A familiarization-novelty technique is being used as a method of detecting discriminability. Infants sit upright in an infant seat facing two target chambers displaced approximately 20° to either side of midline. A frontal rectangle will be exposed in each chamber for a two minute familiarization period. Following familiarization, one of the stimuli will be changed either to a backward tilted rectangle (which projects retinally as a trapezoid) or to a forward tilted trapezoid (which projects retinally as the same size rectangle). To the extent that the infant discriminates the novel and familiarized stimuli, he will look longer at the novel one, and thus provide an indication of whether he perceives retinally or objectively (i.e., in terms of shape constancy).

Major Findings: We have just completed apparatus for this project and are currently commencing testing of infant subjects. The apparatus makes use of a TV photographic procedure for recording the infants direction of fixation, and allows for flexible presentation of stimuli and automatic recording of looking time.

Scientific Significance to Mental Health Research Program: The findings of both projects should contribute to our knowledge of the perpetual and intellective capacities of young infants, to our understanding of the role of environmental arrangements in promoting early perceptual development, to the refinement of methodology for studying infant perception and learning, and, ultimately, to the establishment of a diagnostic, remedial, and educational technology which is appropriate and viable early in life.

Proposed Course of Project: This project is an indefinitely continuing one in pursuance of the general objectives stated above.

Honors and Awards: None

Publications:

Caron, A.J.: Review of A.V. Zaporozhets, and D.B. Elkonin, The psychology of preschool children. Contemp. Psychol. 18: 539-540, 1973.

Caron, A.J., Caron, R.F., Caldwell, R.C., and Weiss, S.J.: Infant perception of the structural properties of the face. Develpm. Psychol. 9: 385-399, 1973.

Serial No. M-P-L-12
1. Laboratory of Psychology
2. Section on Perception
3. Bethesda

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cortical Mechanisms in Somesthesis

Previous Serial Number: M-P-B-12

Principal Investigator: Josephine Semmes

Other Investigators: Blair Turner, Ph.D., Howard University, Guest Worker

Cooperating Units: None

Man Years

Total:	3
Professional:	1 1/3
Other:	1 2/3

Project Description:

Objectives: The general aim is to identify in monkeys the neural mechanisms concerned in various kinds of somesthetic perception, with especial reference, this year, to the functions and interrelations of subdivisions of the parietal lobe. Recent experimentation has focussed on three such subdivisions: a) Somatosensory Area II (SII), as defined electrophysiologically by Woolsey, occupying part of the parietal operculum; b) the posterior part of the postcentral gyrus, which is part of Woolsey's Somatosensory Area I (SI); and c) the part of the parietal lobe lying posterior to SI and anterior to the prestriate region. The immediate purpose was to define the behavioral significance of each of these subdivisions and to determine in this way where functional boundaries could be drawn. Since anatomical and electrophysiological studies nearly always lead to hypotheses concerning function, behavioral tests are needed in order to confirm, deny, or modify these hypotheses.

Methods: Rhesus monkeys are trained to perform somesthetic discrimination tasks requiring them to reach with one hand into an opaque box and select one of two stimulus objects differing in some way and to indicate their choice by pulling the object toward them. For each pair of objects, one (randomly shifted in position) is consistently rewarded whereas the other is never rewarded. Discriminations tested in this situation have involved consistency, shape at different levels of difficulty, size, roughness, and temperature, with each of the last three followed by repeated ascending and descending threshold determinations. Tests in which the subject is confined to a primate chair and required to respond to externally-applied stimuli, and tests of competency in motor performances, have also been used.

Ablations of the selected cortical areas are made by gentle aspiration and are, as far as possible, restricted to grey matter. Aseptic techniques, barbiturate anesthesia, and antibiotics (topical and systemic) are routinely employed. The postoperative recovery period is usually four weeks, after which adaptation and training are begun. Following completion of the behavioral observations and formal tests, the animal is given a lethal dose of barbiturate, and his brain is processed for histological examination. The actual limits (and depth) of the cortical lesion, as well as the consequent degeneration of parts of the thalamus, are then determined. Special attention is given to unintended sparing of tissue within the limits of the planned ablation and to unintended damage of grey matter outside these limits or of fibers passing underneath to areas other than that ablated.

Major Findings: The most important and clear-cut discovery in the past year concerns Somatosensory Area II. Although other investigators have failed to obtain impairment following removal of this region, we have found moderately severe deficit on many of the tasks of our battery. The impairment differs in several ways from the one we reported in previous years as characteristic of animals with Somatosensory Area I lesions: (1) a bilateral lesion is necessary to produce the SII deficit, whereas a unilateral lesion (opposite the hand tested) is sufficient to produce the SI syndrome; (2) the SII deficit is less severe than that associated with SI; and (3) the SII deficit, unlike the SI impairment, is not manifest on discriminations requiring the greatest palpatory activity by the subject. These findings are in harmony with the differences between SI and SII shown anatomically and electrophysiologically, i.e., the sensory projection to SII is bilateral and relatively sparse, whereas that to SI is predominantly contralateral and abundant. The findings can also be interpreted as supporting the theory we and others have previously advanced that the primary function of SI is in "active touch," the interplay between sensation and movement, rather than in refinements of sensation.

With respect to ablations of the posterior part of the postcentral gyrus, we have attempted to find out whether or not the deficit we reported earlier, i.e., a selective impairment in shape discrimination, resulted from removing the part on the crown of the gyrus or the part in the depths of the intraparietal sulcus. We found that neither of these partial lesions was sufficient to produce the deficit; therefore, we concluded that the total area removed earlier (area 2 of Brodmann, defined cytoarchitecturally) is probably unitary, rather than composed of two subareas of different potency.

Regarding the third area on which we have concentrated, the posterior parietal region (the part of the parietal lobe behind area 2), we have so far been unable to obtain the impairment reported by many other investigators. Since we made two different lesions of this region (the posterior bank of the intraparietal sulcus in one group and the crown of the inferior parietal gyrus in another), it is probable that the lesions were too small to produce the characteristic deficit, and hence that this region also must be regarded as unitary, at least from the point of view of its involvement in somatosensory discrimination. Our inability to obtain the posterior parietal deficit with the tests and lesions we have employed up to now precludes the direct and detailed comparison that we hoped to make between that and the impairment we

have found to be characteristic of lesions of the posterior part of the postcentral gyrus. It is clearly necessary to compare the effects of complete lesions of each of these two regions.

Besides these studies primarily aimed at revealing how the cortex should be divided functionally, we have also pursued our studies of temperature discrimination. We have found that (unlike consistency, shape, size, and roughness), fine gradations of temperature can be accurately perceived by the monkey in the presence of large lesions of the sensorimotor cortex. Our studies, this year, have explored the effects of a great number of different parietal removals, with the aim of discovering the smallest lesion that will cause a detectable deficit. (This deficit appears in the number of postoperative trials required to reach a threshold of below 2°C, rather than in a frank elevation of thresholds.) So far, it appears that the smallest lesion that will produce this deficit is a bilateral one: SI on both sides, and SII in at least one hemisphere. This unexpected result emphasized that temperature (like light tough) is not highly dependent on the cortex (in particular the sensory areas) and hence that it must be considered differently from the other, more cortically-dependent, somatosensory submodalities. We are interested in pursuing studies of temperature discrimination, not only for their own intrinsic interest, but because temperature is probably closely similar to pain in the mechanisms governing it.

Dr. Turner is attempting to find out if he can reproduce the severe sensory deficit (e.g., in consistency, shape, and roughness),which we have found to be associated with lesions of the postcentral gyrus, by taking out the four cortical areas downstream from it, to which it sends a heavy projection. No results can be reported for this experiment so far.

Scientific Significance to Bio-Medical Research: An understanding of how the brain processes its afferent inflow is the first step in understanding the neural basis of higher mental functions, such as complex perceptions and thought. The major mental disorders, as well as some diseases within the province of neurology, are, or include, disturbances of such higher functions. Although it is conceivable that the pathology in these conditions is confined to the uppermost levels of processing, it is at least equally possible that the simpler levels are affected and hence that the brain's higher organizing systems are deranged because they are constantly receiving abnormal inputs from the lower systems.

Future Course of Project: We hope to sort out the functionally-meaningful subdivisions of the sensory cortex and to determine the particular connections that give each its unique character. There are several issues that we should like to deal with eventually: (1) the problem of perceptual synthesis, or how somatic submodalities combine to make possible recognition of objects; (2) the relationship between lower and higher functions (in the case of the parietal lobe, this might mean, for example, how somatosensory discrimination is related to spatial behavior and thought); and (3) the sequence of events in the brain between the registration of a stimulus and the organism's motor response. We are still very far from being able to come to grips with any of these issues; we hope, however, that the information obtained in our experiments will be useful in the slow accretion of the relevant body of knowledge.

Honors and Awards:

1. Professional Achievement Award, 1973, The University of Chicago Alumni Association (national).

2. Consulting Editorship, Journal of Comparative and Physiological Psychology, 1974 - .

3. Editorship, Cortex, 1968 - .

Publications:

Semmes, J.: Somesthetic effects of damage to the central nervous system. In Iggo, A. (ed.): Handbook of Sensory Physiology, Vol. II. New York, Springer-Verlag, 1973, pp. 719-742.

Semmes, J., Porter, L., and Randolph, M. C.: Further studies of anterior postcentral lesions in the monkey. Cortex, in press, 1974.

Porter, L. and Semmes, J. : Preservation of cutaneous temperature sensitivity after ablation of sensory cortex in monkeys. Exptl. Neurol. 42: 206-219, 1974.

Randolph, M. C. and Semmes, J.: Behavioral consequences of selective subtotal ablations of sensory cortex in Macaca mulatta. Brain Research, in press, 1974.

Serial No. M-S-C-11
1. Socio-environmental Studies
2. Office of the Chief
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Social Psychological Correlates of Occupational Position

Previous Serial Number: Same

Principal Investigators: Melvin L. Kohn and Carmi Schooler

Other Investigators: None

Cooperating Units: None

Person Years

Total:	5-1/6
Professional:	1-3/4
Other:	3-5/12

Project Description:

Objectives: To ascertain the relationship of some principal dimensions of job, occupation, and career to relevant aspects of men's values, social orientation, self-conception, and off-the-job behavior.

Methods Employed: Structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations in the United States, conducted in the spring of 1964.

Major Findings: The major accomplishment of the year has been the planning and development of a follow-up survey, designed for reinterviewing a subsample of 750 of the men interviewed ten years ago and, this time, their wives and in some families a child as well. Since the follow-up study represents a major new stage in this research program, it is appropriate to review the earlier stages of the research and the rationale for the new survey.

The research began, nearly two decades ago, with a study in Washington, D. C., addressed to confirming earlier reports about class differences in parents' child-rearing practices and to discovering whether these differences in parental practices could be traced to class differences in what parents value for their children. The research showed that social class position is significantly related to both fathers' and mothers' values for their children,

with parents of higher social class position valuing self-direction more highly, and parents of lower social class position valuing conformity to external authority. Moreover, these class differences in parental values have important consequences for their child-rearing practices, both for disciplinary practices and for the division of responsibility, between mother and father, for providing support and for imposing constraints on the children.

A second study, in Turin, Italy, showed that social class position has essentially the same effects on parental values in the very different historical, cultural, and economic circumstances of the United States and Italy. This study also indicated that class differences in fathers' values (and to a lesser but still substantial degree, in their wives' values) could be interpreted largely in terms of class-related differences in men's occupational conditions, particularly those involved in the exercise of self-direction in work--notably closeness of supervision, routinization, and the substantive complexity of the work.

A third study--this one a survey in 1964 of a cross-section of all men employed in civilian occupations throughout the United States--made it possible to extend these findings in several important respects. First, these data demonstrated a remarkable generality to the relationship between class and paternal values: the relationship exists and is of approximately equal strength in all segments of American society. Second, these data show social class to be more strongly related to paternal values than is any other major line of social demarcation, more strongly in fact than all other major lines of social demarcation combined. Third, the importance of occupational conditions for explaining the relationship between social class and paternal values is much more firmly established by these data than by those from Turin. And finally, these data show that class and occupation are related not only to parents' values for their children, but also and in exactly the same way to their values for themselves, to their self-conception, and to their social orientations.

These findings establish a prima facie case that men's social class position affects their values and orientation, at least in substantial part, because of class-related conditions in their occupational lives. But this explanation assumes that the interrelationship of class, occupation, and values is not just a function of what types of men go into what types of jobs, and of men's efforts to mold their jobs to fit their needs and values. In further analysis, borrowing from the analytic techniques of the econometricians, it has been possible to show that the effects are not all one way: that the job does affect the man; the analysis suggests, in fact, that the effects of job on man are stronger than are the reciprocal effects of selective recruitment and job-molding.

Even the application of econometric techniques to the analysis of the reciprocal effects of job on man and man on job cannot definitively show change in particular individuals. Nor does it provide much information about the learning mechanisms involved in transferring the lessons of the job to nonoccupational realms of life. To accomplish these goals requires a follow-up study of a substantial subgroup of the original sample, inventorying their current job conditions, recording all changes in their occupational conditions since the original survey in 1964, and re-measuring all the principal facets of psychological functioning examined in the earlier study. This is the study now being conducted.

The second main objective of the follow-up study is to interview the wives of all those men who are currently married, using interview procedures closely parallel to those used in the interviews with the men. There are three principal reasons for interviewing the wives.

One reason is to fill in a major gap in our work thus far: since our original survey was limited to men, our inquiries to date tell us nothing about the effects of occupational conditions on the psychological functioning of women. Admittedly, our sample of wives is not optimum for this purpose, not only because a sample of wives is not representative of all women, but also because the wives in our sample are all married to men who are between the ages of 26 and 65. Nevertheless, if these sample biases are kept firmly in mind in analyzing and interpreting the data, much can be learned from such an inquiry.

A second reason for interviewing the wives is to examine the relationship between men's occupational conditions and their wives' psychological functioning, as well as that between the wives' occupational conditions and their husbands' psychological functioning. The Turin study showed that men's occupational conditions are meaningfully and apparently causally related to their wives' values. But this research gave no information about the processes involved, nor did it examine the corresponding effects of wives' occupational experiences on men's values and psychological functioning. Parallel interviews with husbands and wives should enable us to do both.

The third reason for interviewing wives is that we want to study the processes of "value-transmission" from parents to children. Such an analysis requires information from both parents, with respect to both parents' experiences, both parents' values, and both parents' perceptions of family relationships.

For all three purposes, we need data on the wives' occupational experiences. A major problem here, of course, is that many wives do not have jobs outside the home. But most "unemployed" wives do a

substantial amount of household work, and some work in voluntary activities. We are trying to assess these activities in the same terms as other jobs. We are dealing with household work as an "occupation," to be studied in much the same way as other occupations.

The third and final objective of the follow-up study is a survey of the children of some of the men interviewed in 1964. In the 1964 survey, all questions about parental values and child-rearing practices were asked with reference to one specified child, who had been randomly selected by procedures designed to give an approximately equal distribution of boys and girls at every age level from 3 through 15. This specific child is to be the subject of the children's part of the present inquiry. Thus, the age range of the children, ten years later, will be 13 through 25. Our intent is to try to interview these children wherever they now live, wherever they may be in their educational, occupational, and family careers.

Our earlier studies provide evidence for a causal chain of considerable importance in understanding the personality development of children: from parents' social class position and attendant occupational experiences to parental values to parental practices. What is lacking is the next and crucial link in this causal chain: from parental values and childrearing practices to the psychological development of the child. The data on paternal values and child-rearing practices collected ten years ago, together with the data now to be collected from a subsample of these fathers and their wives, provides an excellent opportunity to study this crucial link in the causal chain. The critical interpretive problems in past studies of what has come to be called "the transmission of values in the family" has been the lack of relevant time-series data. The planned study of the children should enable us to deal with this problem directly.

In addition to the questions asked of parents, two new sets of questions have been developed for the children's study. The first concerns parent-child relationships and identification with the parents. The primary intent here is to develop indices that can be used as intervening variables, in explicating the processes by which parental experience influences children's values and psychological functioning. That is, we hypothesize that the effect of father's experiences and values on child's values will be mediated by the nature of the father-child relationship and by the degree of identification of child with father; similarly for the mother-child relationship.

The other new element in the inquiry with the children is a set of questions on their educational experiences, to be asked of

all children who are still in school. Since the amount of interview time available is too limited for us to explore all facets of the educational experience, we chose to concentrate on those that parallel the main dimensions of occupational experience: the degree of self-direction and of intellectual flexibility permitted and encouraged by the school experience. This, too, represents a new departure, and we are pleased that in the pre-tests the questions seemed to work well with students at all levels, from junior high school through graduate school.

Scientific Significance: In industrial society, occupational role is of central importance in shaping men's conceptions of self and of social reality. This research is addressed to a systematic appraisal of precisely which aspects of occupation affect which facets of man's social psychological functioning.

Proposed Course of Project: The main survey is to be conducted in the late spring and early summer of 1974. Processing the data for computer analysis will take much of Fiscal Year 1975. Thereupon, we anticipate spending several years on the analysis and interpretation of this immensely valuable body of data.

Honors and Awards: Melvin L. Kohn: elected to the governing Council of the American Sociological Association; elected Vice President of the Society for the Study of Social Problems.

Publications: None

Serial No. M-S-D-10
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Research on the Processes of Internalization of Rules, Standards, and Values

Previous Serial Number: Same

Principal Investigator: Roger V. Burton

Other Investigators: None

Cooperating Units: None

Person Years

Total:	2-1/4
Professional:	1
Other:	1-1/4

Project Description:

Objectives: The current interest of this research is on the way that cognitive factors in training and instructing children in self-control influence the acquisition and performance of honesty. Previous studies have shown that although honesty is very much situationally determined, the evidence also demonstrates that there is a modest generalized tendency within individuals to react similarly across different temptation situations. Of greater immediate interest is the evidence that consistency of behavior across situations varies among individuals. Thus some persons are unpredictable from one situation to another, whereas others are consistently deceitful.

To account for these individual differences in consistency, it has been proposed that two facets of learning are involved in behavior elicited during temptation and that these determine the extent to which there is transfer (or generalization) from situation to situation. The first facet encompasses factors limited essentially to the immediate external physical situation. Examples of these factors are the apparent risk involved in whether the person is alone or another is present, the location (home, school, playground), etc.

The second facet involves the way the person conceptually classifies the situation. When a child learns that a particular response is correct or prescribed for a specific situation, he or she may also learn that this situation is an instance of a particular concept, in these studies the concept of honesty. This research is now exploring how behavior learned for a specific honesty situation might be linked to the concept of honesty in such a way that subsequent behavior in other quite distinct kinds of honesty tests becomes more consistently honest. In other words, the objective is to demonstrate how use of a general conceptual label in teaching honesty in a specific instance may mediate behavior in other, physically distinct, but conceptually similar situations.

The theoretical model guiding this study is that consistency of conduct is determined by both physical (primary) stimulus generalization and verbal or cognitive generalization. It is cognitive generalization that permits physically different situations to be perceived as similar. Acquisition of such cognitive generalization should vary according to the degree of generality of the verbal labels (concepts) used in training. The attempt, then, is to establish sets of instruction that vary in degrees of generality of critical labels used that then produce reliable differences in honest conduct. It was predicted that minimum cognitive structure is provided by telling the child simply to "do it like this" or "don't do that." Reliance on such instructions should produce little transfer to other situations. The hypothesized order for increasingly more hierarchically inclusive terms for the different sets of instructions was from "rules," then "cheating," to "honesty."

Conceptions of Honesty. The first task was to explore what concepts children of varying ages used for classifying honesty tests and to establish when children could classify events hierarchically. We also needed some evidence of what the order of critical terms was empirically as well as theoretically. In this phase of pretesting, cartoons depicting a child committing a deviation were used to question children about their concepts of moral conduct. The 30 settings used were varied and included classroom tests, eating forbidden candy, playing games of skill, and games of chance. We found that in a free-responding format the children, from ages 5 to 10, did not use the terms "honest(y)" or "dishonest(y)" to describe what was depicted by the cartoon. The youngest children (5 and 6) tended to describe the act as, "not right," "wrong," or "not fair." From 7 on, the children tended to use "cheating" to describe the situation.

The children were then asked to group the different cartoons any way they might go together. The younger children tended to form groups on the basis of external quality or functional features, such as "these have round things" or "these have things that can

be written on." The use of more abstract conceptual categories, such as "these are stealing" and "these are games," increased with age.

Finally the children were asked to put together all the cartoons that fit a particular term or concept. The terms and concepts were "things done in school," "games involving rules," "lying," "cheating," and "things involving honesty or dishonesty."

These data indicated the following: (1) before age 7, many children are unable to classify these conduct situations by hierarchically more inclusive concepts and are not yet clear about the meaning of honesty even when directly asked. For most of these children, "cheating" is a more inclusive term than "honesty"; (2) "honesty" is used by the 7-year-olds for classifying most of the situations, but the number of exceptions indicates there is still some confusion about the term; (3) 9-10 year-olds seem to have no problem in grouping the situations by more inclusive terms and in perceiving that all the situations involve "honesty"; (4) the hypothesized hierarchical ordering of terms received some support, especially in the older groups of children.

These data suggested that 7-year-olds are acquainted with the critical terms we considered worthwhile to try (rules, cheating, honesty) and when forced are able to classify the situations by these superordinate moral labels. Yet, from the free-responding and grouping data, they still appear to be confused and vague about using these terms on their own. Since the objective is to develop a procedure that might show the connections between acquiring the concept of honesty and acting honestly, we decided to use 7-year-olds for the next phase of pretesting since they seem to be in a transitional stage in acquiring a clear understanding of the concept.

Development of Procedure for Teaching Generalized Honesty. From the pool of 30 honesty tests, 11 tests have been selected as most suitable for this study. The tests involve games of skill, material gain, delay of gratification, peeping, academic tests, and puzzles. With one exception (a delay of gratification test in which the child is not to touch attractive toys) there are two similar tests to assess each type of honesty.

The procedure developed entails administering 5 tests to a child to establish a baseline of the child's general tendency to conform to rules in these situations. The rules are simple in order to eliminate ambiguity in understanding or learning them. The instructions used to teach the rules on these baseline tests are the same for all children.

The experimental treatments are administered the second day. Each child is assigned to one of four sets of instructions

that vary from minimal cognitive structure provided for transfer ("do this," or "don't do that") to use of the critical term of "honest," which hypothetically should maximize consistency of behavior in the various tests. The other two levels of instructions use "rules of a game" and "cheating" as the critical terms. To control for differences in baseline consistency, the number of baseline tests in which rules were broken is used to assign the child to one of the four experimental groups. (The four treatments [sets of instructions] are also balanced for sex of child and sex of experimenter to control for possible sex differences since they are not of major interest at this time.)

The experimental treatments are given as the instructions for the first two tasks of the second day. After the instructions, the child is asked to tell the experimenter what he/she is to do and then why. The child is guided to use the critical term to insure that the child has attended to it.

Following these two tasks are four more tests. The style of instructions is identical for all children on these remaining tasks, again providing a minimum of cognitive structure as was true for the first test day.

A day or two later, an interview is administered that assesses the child's conceptions of these tests. Cartoons of the tests are used as stimulus materials for asking the children to group the tests by the critical terms used in the different sets of instructions. Measures of the importance of doing well on the different tests and of the child's level of moral judgment are also obtained. These data serve both as controls for the impact of the child's cognitive structure on consistency of following rules and as measures for assessing the effects of the instructions on the child's conceptions of moral conduct situations.

When testing of children in a school is finished, they are rewarded by receiving either a small candy bar available immediately or, if they are willing to wait, a larger candy bar "next week sometime." Their choice provides the children with a reward for their participation and the experimenter with an assessment of the child's ability to delay immediate gratification.

Analyses of these pretest data demonstrate that the tests discriminate well and are reliable. An exception is for the two tests that involve taking pennies or eating candy, situations that elicit very little deviation among these pretest children. Nevertheless, since these tests assess an important facet of honesty, are economically administered, and permit an unobtrusive measure of achievement motivation, they are retained.

During this pretesting, a script has been developed for instructing each game and each experimental treatment level. Also reliable observational measures have been developed that are theoretically relevant and yet come from the natural setting. Examples of such measures are the child's use of self-instruction when performing (e.g., saying aloud what score is to be recorded), self-assessment of performance when the adult returns following a test, and his manifest involvement in the activity. The major measures of whether there is rule infraction, time of first infraction, frequency of infractions, and total time engaged in the activity are measures previously established in controlled experiments to be sensitive for these tests.

This part of pretesting has passed through three stages. The first was the original exploring of the children's reactions to the various tests, ways of presenting the tests, etc. The second phase employed a first attempt to codify a script and a set of tests. Data from this phase indicated changes in the presentation of the tests, in the procedures of some tests, and in the complete substitution of some tests were called for. The third phase used a procedure that seems promising for further testing. Data from these pretests are being used to develop appropriate analytic models.

Preliminary analyses indicate that the different treatments have a marked effect on the behavior of the children. The interpretation of these effects, however, are tenuous because the data are skewed in a special way (the statistical term is "censored data"). A recently developed statistical model is being applied to the data to see if the problems can be eliminated.

A very preliminary suggestion from these analyses is that the use of "cheating" as the critical term in the instructions has the most impact on increasing consistency of honesty. Should this result replicate, it may be due to the indication that this is the most meaningful concept for deterring deviation for these children as suggested by its being the most common term they use to describe infractions. Perhaps "honesty" as a term is too detached to serve these children as an effective mediator for arousing affect during temptation, whereas "cheating" is especially effective in eliciting affective arousal.

Methods Employed: The 11 behavioral tests are the same or modified versions of measures used extensively in previous studies of honesty. These tests involve simple rules, similar to rules the children experience daily. For example, the arithmetic test consists of 15 problems of increasing difficulty that the child is to complete in 2 minutes. The Initial problems are very easy but quickly become difficult so that it is unlikely that any child can complete all the problems correctly in the allotted time. The child begins the test when the

experimenter sets a 2-minute timer and leaves the child in the room alone. When the timer rings, the child opens a folder with the answers and checks his own test. After 3 more minutes, the experimenter returns. The child may deviate from the rules by looking at the answers before the timer rings, changing or adding answers after the allowed time, and recording more correct items than there should be. Observers behind a one-way mirror score the time and number of the item if and when the first infraction occurs, the frequency of infractions, and record other potentially relevant material.

The assessment of the child's cognitive orientation of moral conduct is obtained through a semi-structured interview with cartoon drawings depicting the test situations as stimuli. The child's responses are scored according to pre-coded categories.

Major Findings: Only the pretest data are in, and the tentative findings have been mentioned.

Scientific Significance: Most previous work in moral conduct has explored single tests of honesty. Little research has explored factors influencing consistency of honesty since 1928 when Hartshorne and May came to the conclusion that honesty was primarily situationally specific. Nevertheless, their work provided the evidence for some consistency in moral conduct and especially for individual differences in such consistency. The teaching of self-control is basic to society's survival and is a major task in socializing a child. The demonstration of teaching techniques that can facilitate and enhance the acquisition of generalized moral conduct would contribute both practical and theoretical knowledge about the socialization process.

Proposed Course of Project: The procedure that has been developed permits us to extend the ages of children tested. At this time we plan to test from kindergarten ages (5) to third graders (8-9). In addition, when the children have finished the testing described, we shall ask their mothers to serve as instructors in a new test. This addition will provide some indication of the customary techniques the child has experienced in being taught to abide by rules.

Honors and Awards: Program Chairperson for Div. 7 (Developmental) of American Psychological Association for the 1974 program.

Selected to present the G. Stanley Hall Award to J. W. M. Whiting at the 1973 American Psychological Association meetings.

Publications:

Burton, R. V.: Folk theory and the incest taboo. Ethos, 1: 504-516, 1973.

Serial No. M-S-D-26
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: An Observational Study of Maternal Models

Previous Serial Number: Same

Principal Investigators: Carolyn Zahn Waxler and Marian Radke Yarrow

Other Investigators: None

Cooperating Units: National Institute of Child Health and Human Development

Person Years

Total:	5/12
Professional:	5/12
Other:	0

Project Description:

Objectives: To investigate, under semi-naturalistic conditions, variations in maternal modeling in relation to children's imitative learning. The purpose is: (1) to ascertain how clearly in evidence are certain of the relations that have been demonstrated under laboratory conditions, specifically concerning reinforcement and the origins of imitation; and (2) to attempt to identify critical dimensions of mother-child interaction that affect imitation.

Methods Employed: Thirty-five black mothers from middle and lower middle economic classes were observed with their 20-month-old children. Observations were carried out, in a laboratory trailer, during a brief waiting period, in exploration of a new play environment, and in a planned modeling setting. Acts that the mother had performed were later repeated by an experimenter and the child's responses to the experimenter's modeling were observed. Interactions were recorded, with the mothers' knowledge, from behind a one-way screen. Observer reliabilities on exact matching of sequential behavior units range from 68% to 87%.

Major Findings: What characteristics of the mothers are associated with different levels and kinds of imitation in the children?

1. Mothers who reward or praise imitation have children who imitate more frequently than children of mothers whose rewards are non-existent or minimal. This association exists within a given situation. However, reinforcement or lack of it does not influence the performance of new and different (generalized) imitations. Mother's reinforcement characteristics in a half hour of interaction with her child do not predict the levels of the child's imitation in a later period in which the mother is asked specifically to provide unique behaviors which the child can match. Reinforcement provides a partial explanation for how frequently the child copies, but not for the pervasiveness of his imitation. This is at odds with the experimental research; and it raises methodological and substantive questions about hypothesis testing in the laboratory and in field work.

2. Other characteristics of mothers as models are associated with children's imitation. Mothers' behavior was coded in terms of the kinds of actions she presented and how she portrayed them. Acts were coded as discrete or thematic; content as nurturant, playful, or aggressive. Mothers' modeled acts were coded as "unadorned," highlighted, invitational, attention-demanded. Variety and repetitive qualities were also coded.

(a) Highlighting and variety in modeling are associated with more and more varied imitation.

(b) Unadorned modeled acts and demanded attention result in lower frequencies of imitation.

(c) Thematic content is not more likely to be imitated by these very young children than are trivial discrete acts.

(d) Acts that mothers continue to repeat in stereotyped fashion tend not to be selected for imitation by the child.

(e) There appears to be an optimal amount of modeling. Very few and very many presentations by the mother are associated with low imitation by the child. This seems to be inappropriate "pacing" by the models.

(f) Child effects cannot be ignored in the associations between what models do and what children imitate. Factors of child's mood, competing interests, importance of contacts with mother influence the amount of child's imitation and also the way in which the mother conducts herself.

From the entire set of findings, a configuration of an effective model begins to emerge. A single dimension does not seem to control the variation in children's imitative responding.

Scientific Significance: Before applying principles that are derived from laboratory work to practices in child care, it is essential to examine such principles under more nearly natural conditions. Such re-examinations validate or modify theoretical formulations and also provide leads for further research.

Proposed Course of Project: The analyses will be completed in the next several months; a manuscript is now in preparation.

Honors and Awards: None

Publications:

Yarrow, M. R.: Research on child rearing as a basis for practice. Mental Health Digest 5: 1-6, 1973.

Serial No. M-S-D-28
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A Comparison of Methods of Obtaining Data on Parent and Child Behavior

Previous Serial Number: Same

Principal Investigators: Marian Radke Yarrow and Carolyn Zahn Waxler

Other Investigators: None

Cooperating Units: None

Person Years

Total:	1/12
Professional:	1/12
Other:	0

Project Description:

Objectives: Observing behavior is remarkably difficult if one requires of the procedure the same standards of good measurement that are required of other scientific tools. The observational data in behavioral research derive from diverse philosophies, techniques, and degrees of rigor. The resulting raw data are, therefore, highly variable in form, specificity, and reliability. The purpose of this research is to examine some of the influences of observing techniques on the kind of data obtained.

1. How well are different techniques of observation suited to different research purposes? How does the form in which observations are recorded limit the kinds of analyses that can legitimately be made?

2. How reliably can observations of different forms, about different behaviors, and in different circumstances be made?

In this research we are examining our cumulative research experience over the past ten years with regard to the varied observational techniques that we have used in our Program in Developmental Psychology, to contribute some answers to the preceding questions of efficacy and reliability.

Methods Employed: Sample: Approximately 300 pre-nursery school and nursery school aged children were the subjects. About 150 were observed with their mothers; the others were child-teacher and child-child interactions.

Procedures: Six studies are the basis of these analyses. The designs involve naturalistic observing in homes and nursery schools, observations in quasi-naturalistic settings, and highly standardized experimental social situations involving single pairs of participants. In some of the studies, verbal reports from mothers were another source of data that can be compared with observational records.

Major Findings: The following are selected findings relating to observing methods and reliability: A procedure was developed by which was recorded an extensive range of environmental events and interpersonal behaviors in their temporal relation to each other. This kind of data is needed, ideally, if one is to investigate contingent relations, interrelations of various behavioral dimensions, and contextual influences on behavior. Observers dictated ongoing events, with a single child as the focus. In observing and coding categories of low levels of inference were used. The records of independent observer-coders were compared to ascertain the feasibility of the method.

1. Under completely naturalistic conditions sequential reliabilities are consistently low, too low to be useful. This criterion of reliability is very demanding, requiring that the same pieces of behavior are reported by two observers with the same kind of unitizing and in the same temporal relation to other pieces of behavior.

The sources of disagreement in the resulting documents were studied: (a) The major source is not coding. When a single observation record is coded by two trained persons, agreements are acceptable. (b) Even when item by item analyses have low interobserver agreement, when the independent accounts of pairs of trained observers are read, the accounts sound very similar, and result in similar ratings and clinical assessments. Disagreements in the item analyses can be traced to: (1) differences in narrative styles which result in breaking up the behavior into different kinds or sizes of units, (2) changes in the order of reported acts, and (3) differential omissions of acts by two observers. By developing an observing language style, short of pre-coding, some of these recording differences can be reduced.

The low agreement levels particularly in relation to sequential analyses of naturalistic data raise serious questions about the prevalent research practice of using a single observer protocol and attending only to coder reliability.

2. These same naturalistic observation records can be rendered "reliable" if, instead of requiring point by point agreement between data analysts, one considers only the frequencies with which observers report given categories within given time periods. Apparently different observers are sampling behaviors similarly but are not necessarily reporting all of the same behaviors. Reliability based on frequencies limits use of the data to research questions that do not involve issues of temporal order.

3. When the narrative observational methods, subjected to sequential analyses, is used in social situations on which some degree of experimental control has been imposed, sequential reliabilities are improved to acceptable levels. This would indicate that observers can be trained to process and record a wide range of behaviors if there is some narrowing of the range of possible events, and if some expectations are given to the observers regarding the framework in which they are to observe. A very considerable amount of observer training is necessary. Under these circumstances it is not necessary to resort to the severe restrictions on analysis that follow from observation methods that use time sampling, simply noting the presence or absence of given behaviors in successive time blocks. The latter methods destroy continuities in interaction, and potentially distort the record of behavior.

Scientific Significance: Methodological improvements are of fundamental importance in behavioral investigations. The development of standard approaches, with high levels of efficiency and reliability would do much to bring about greater comparability among studies and to reduce variable sources of error in the raw data of research.

Proposed Course of Project: An increasing amount of staff time will be given to this project in the later part of the coming year. Analyses extend into issues of observer influences, comparison of assessments of the same variables across methods, and issues of conceptual levels in observing.

Honors and Awards: None

Publications: None

Serial No. M-S-D-29
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Development of Sensitivity to the Feeling-States of Others

Previous Serial Number: Same

Principal Investigators: Marian Radke Yarrow, Carolyn Zahn Waxler
Robert A. King

Other Investigators: Judith Brady, C. Jean Darby, Carol Jones, Marilyn T. Pickett, Frances L. Polen, Delores Walker

Cooperating Units: None

Person Years

Total:	4 2/3
Professional:	1 5/6
Other:	2 5/6

Project Description:

Objectives: The research focus is on the beginnings and the evolution of children's sensitivities and responsiveness to the affective or emotional states of other persons. The problem is approached longitudinally over the second and third years of life. How much do the psychological states of others enter into the very young child's awareness and into his own feeling-states? What are the patterned developmental regularities in signs of recognition and in qualitative responses to the affects of others? How are other dimensions of psychological development and differences in rearing conditions related to variations in children's reactions in this sphere?

Methods Employed: Eight mother-infant pairs were selected at each of the following ages: 9-10 months, 15-16 months, and 21-22 months. The families are a volunteer, middle to upper middle class, suburban sample. The families are observed over a 9-month pericd.

Data on the child's responses (or lack of) to the feeling-states and intentions of others are obtained from two sources:

(1) The mothers.--Given training in the procedures to be used, and provided with a reporting guide, mothers dictate accounts of naturally occurring events in the daily lives of the children. The relevant events are situations in which feelings or emotions are expressed by someone in the presence of the child. Additionally, in semi-standardized fashion, the mother or father enacts specified feelings (fatigue, pain, affection, sadness, anger, enjoyment). The circumstances of these natural and the experimental events are reported, along with the child's reactions, and any subsequent responses that are made to the child. At 3-week intervals mother and researcher meet to review the mother's records. (2) The researcher.--When the staff member visits the home, she not only keeps the mothers "on course," she also obtains additional data by (a) carrying out semi-standardized incidents in which she expresses affect (enjoyment, physical pain, and anger), and (b) obtaining interview and observational data on related aspects of the child's development and on mother-child interactions. (c) After the visits, the researcher dictates a clinical assessment of a number of dimensions of child and mother behavior. Mothers' and researchers' styles and reliability of reporting are assessed by comparing mother's and researcher's accounts of the same standard affect-situations carried out by the researcher during the home visits. A second researcher is also present on some visits to provide an additional standard.

A major analytic framework is descriptive, maturational. A second analytic direction focuses on the rearing variables associated with variations in children's responsiveness to others' affects.

Major Findings: Data collection began in April 1973. Although most of the data have been collected, analysis is at very early stages.

Scientific Significance: Developmental psychology is without a base of empirical data regarding the child's earliest abilities to comprehend the attributes or states of others. There are few systematic data on the early indicators of normal or pathological development. An understanding of early self-other differentiations and of the development of empathy and role-taking could be advanced by systematic evidence on the child's emerging capabilities in perceiving others. Further, psychopathology often involves significant distortions or limitations in the individual's awareness of other people's feelings and his sense of similarity or difference from others (e.g., childhood autism). Understanding of many forms of psychopathology could be advanced by a more thorough knowledge of the phases of the normal child's evolving sensitivity and responsivity to the feeling states of others.

Proposed Course of Project: Primary data collection will be completed in June 1974; brief follow-ups of the subjects will be made during

the next year. Analysis of data, which has already begun, will become the main emphasis as data collection comes to an end. The data are extensive and do not fall into readily defined categories. Therefore, a major task will be to develop meaningful concepts and categories in an unexplored area of study.

Honors and Awards: None

Publications: None

Serial No. M-S-D-30
1. Socio-environmental Studies
2. Developmental Psychology
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Developmental Studies of Altruism

Previous Serial Number: Same

Principal Investigators: Marian Radke Yarrow and Carolyn Zahn Waxler

Other Investigators: Judith Brady, C. Jean Darby, Marilyn Pickett, Robert King, Delores Walker, Thomas Padrick

Cooperating Units: None

Person Years

Total:	2 7/12
Professional:	2/3
Other:	1 11/12

Project Description:

Objectives: This research focuses on altruism, developmentally considered--children's potentialities for altruism and the ways in which compassionate behaviors are socialized. The investigation of altruism is not separable, however, from underlying questions regarding the child's comprehension of the perspectives and experiences of others. The research extends, therefore, to more general issues: What are the child's abilities to infer another person's perceptual, social and emotional experiences when these differ from his own concurrent experiences: What is the interrelatedness of these cognitive capabilities and the child's inclination to act in behalf of another person? How age-dependent and how training-dependent are the cognitive and behavioral responses: How are the child's altruistic actions interrelated with "incompatible" actions, such as his competitive and aggressive behaviors?

Methods Employed: Sample: The subjects are 90 children, ages 3 to 7 years, of middle class families. About 5% are non-Caucasian.

Procedures: (1) To examine cognitive capacities: A series of experimental problem-solving tasks and games involving gradations of experience, from understanding the physical world of another person to understanding the psychological and interpersonal world of another. For example, does the child comprehend that another

person, viewing or feeling an object from another position, sees or feels it differently than he does (upside down or right side up, or soft or hard)? Does the child comprehend that the same events can result in different states of mind for self and Other, such as different preferences, feelings of success and failure, pleasure or nonpleasure. (2) To observe possible interventions in behalf of the Other: A number of situations are created in which there is reason for helping, sharing with, or comforting another person (aiding in picking up scattered materials, expressing awareness of a physical injury, sharing scarce materials). (3) To assess social interactions under natural conditions: Interactions of the child with his peers and teachers in the work and play are systematically sampled and recorded. Included are behaviors that are attention-seeking, supportive, competitive, aggressive, cooperative, helpful, sharing, sympathetic.

Study B: Training will be undertaken to increase children's sensitivity and responsiveness to others' needs. This part of the research will not be initiated until Study A is completed. Training plans will be influenced by the findings of the first phase of the research.

Major Findings: Systematic analyses are just beginning. Impressions indicate that:

(1) The ability to take the point of view of another person's visual-perceptual perspectives does not appear to develop earlier than the ability to take another person's perspectives in preferences and feelings.

(2) Acts of helping and manifestations of concern for the welfare of others in some instances occur even though the child has been unable to perform the cognitive tasks that require taking the other person's point of view.

Scientific Significance: The child's development with respect to his comprehension of the properties of people and his social environments is as important as his abilities to think and reason and solve problems about the physical, nonsocial world. With respect to the latter, research has greatly expanded our knowledge of the nature of cognitive development, and has established the fact that there are specific developmental patternings. Knowledge in this field has thereby contributed greatly in fields of education and child care by making it possible to provide education and care with much better appreciation of how the provisions are comprehended by children. A similar development of knowledge is needed regarding children's comprehension of persons. Investigation of the individual's humane capabilities has not kept pace with research in areas such as achievement motivation, competitive striving, aggression, power assertion, anxiety, and guilt. Moreover, there

is no compelling evidence to indicate that in the rearing of its children, society has given high priority to the inculcation of personal concern for the welfare of others.

Proposed Course of Project: The collection of data on Study A will be completed by summer, 1974. Analysis will then become the major emphasis. Work on Study B is dependent on the outcomes of Study A.

Honors and Awards: None

Publications:

Yarrow, M. R., Scott, P. M., and Waxler, C. Z.: Learning concern for others. Developmental Psychology, 8: 240-260, 1973. [This article also digested in Mental Health Digest, 5: 2-11, 1973.]

Serial No. M-S-PS-1
1. Socio-environmental Studies
2. Personality and Stress
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Health Orientations of Parents and Children

Previous Serial Number: Same

Principal Investigator: John D. Campbell

Other Investigators: Caren M. Carney

Cooperating Units: Children's Hospital, Washington, D. C.

Person Years

Total:	1-3/4
Professional:	1
Other:	3/4

Project Description:

Objectives: To study health orientations of family members. Specifically, the goals of the study are; first, to describe systematically the patterns of variation in family members' attitudes, values, beliefs, perceptions, and behavior concerning phenomena of illness and health; and second, to examine ways in which selected personal and situational factors relate to health orientations. Relevant to the attainment of these objectives are the following three assumptions basic to the study: (a) an individual's orientation to health and illness is embedded in interactive processes; (b) such orientations are at least in part learned; and (c) to a considerable extent such learning takes place as an aspect of routine family relationships.

Methods Employed: Information on 264 hospitalized children and their mothers was obtained by use of focused interviews, questionnaires, and rating scales. Interviews with children and mothers, and questionnaires completed by the mothers provide data on (a) aspects of their definitions of illness, (b) reports of their own and other family members' feelings and actions when ill, (c) their attitudes and beliefs about illness prevention and treatment, (d) their values in selected areas not directly relevant to health, and (e) views about appropriate standards of behavior: what should or should not be done in circumstances pertaining to illness and health. Ratings, made by nurses in the hospital setting, appraise children's emotional dependence and anxiety.

Major Findings: Central to this research is the question of how health orientations are acquired and modified. Evidence summarized previously indicated that the extent to which children directly adopted their parents' definitions of illness was limited, although some parent-to-child transmission did appear to be the case for the more value-laden area of standards of appropriate behavior in health-relevant circumstances.

Additional analyses show that much of the child's own perspective on health-relevant issues can be viewed as determined by the cumulative impact of (a) the child's own status, (b) the general circumstances of his family, and (c) status-related parental values. The role of these factors is clearly evident in children's assessments of their health-relevant risk taking, their behavior when ill, and their own standards for appropriate behavior in such health-relevant circumstances.

Ascription of status on the basis of age and sex, as Linton long ago pointed out, is common to all societies. At a relatively early age children learn that such status ascription also implies concomitant culturally prescribed standards for behavior. Although the person who is defined as ill may be relieved of certain conventional role obligations, sick-role functioning, nevertheless, exacts its own requirements of the patient. And the nature of such functioning is very much related to age- and sex-typing. Indeed there is a considerable parallel between age and sex in terms of their impact on children's health orientations. Spartan standards for emotional expression when faced with the stressors of illness are characteristic of older children and boys, who say they should display relatively little emotion in such circumstances and characterize their own actions as conforming to these norms. Older children and boys are also more inclined than younger children or girls to reject the sick role (as indicated by a scale measuring preference to keep active, rather than to give in to illness, disinclination to see the doctor, shrugging off minor injuries, and a tendency to curtail the duration of convalescence). In risk-taking behavior, boys and older children also show similarity. They, in contrast to girls or younger children, see themselves as more apt to take risks that might conceivably lead to illness or injury.

An additional powerful predictor of children's health orientations is family socioeconomic status. This probably helps shape children's perspectives on health and illness in an indirect fashion; the social circumstances of the family have a sizeable influence on parents' general childrearing values, and these in turn help determine the climate in which children acquire their health orientations. Several scientists within the Laboratory of Socio-environmental Studies have documented the fact that a family's position within the social structure is linked to parental valuation of self-direction in the child: the higher a family's socioeconomic status, the greater

the likelihood that the parents will attach importance to self-direction; in contrast, children's conformity to externally imposed rules is more apt to be valued by parents at lower status levels. Data from the present study confirm this relationship and show further that socioeconomic status and parental valuation of self-direction make generally parallel contributions to children's health orientations. The higher the social status level or the greater the mother's valuation of self-direction, rather than conformity, the more likely were the children to reject the sick role, to minimize the extent of their emotional reaction to illness, and to be more casual about taking risks that might lead to illness or injury.

Each of the four status-relevant variables considered (age and sex of child, socioeconomic status of family, and status-linked parental valuation of self-direction) made an appreciable independent contribution to children's health orientations. Multiple-regression analyses confirm that their combined effects are considerable. (The average multiple-correlation coefficient showing the relation of these four variables to the dimensions of health orientations here considered was greater than .30; the largest of these was nearly .60.) Thus it appears that the cumulative impact of status-linked cultural prescriptions for role functioning is at least as much in evidence in children's perspectives on health and illness as it is in more general social orientations.

Scientific Significance: This research provides information about a specific area that is currently only inadequately covered; it should lead to a more systematic picture of health orientations and factors associated with such orientations than is now available. The potential value of the research is not, however, restricted solely to the confines of sickness and health. Within the framework of the study, such general social psychological issues as role learning, developmental changes in family relationships, and role behavior in stressful conditions can be treated. The phenomena of illness and health can be viewed as representing specific aspects of these general issues.

Proposed Course of Project: Data analysis continues. Two papers have been submitted for publication. Other research reports will be written after appropriate data analysis.

Honors and Awards: None

Publications: None

Serial No. M-S-S-12
1. Socio-environmental Studies
2. Section on Social Structure
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Developmental Study of the Self-Image

Previous Serial Number: Same

Principal Investigator: Morris Rosenberg

Other Investigators: Roberta G. Simmons (University of Minnesota)

Cooperating Units: None

Person Years

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: To learn how children's self-images change as they grow older and to gain increased understanding of the social factors that influence self-image formation. While most previous systematic research has focused on self-esteem, the present investigation considers a number of other aspects of the self-image as well: degree of self-consciousness; preoccupation with the self; the quality of self-detachment; stability of self-concept; "front" or "presenting self"; committed and idealized images; development of ego-extensions; and other aspects of the self-image. The aim is to understand how these aspects of the self-image develop; to examine their relationships to a range of social conditions and experiences; and to learn how they are associated with other psychological states, such as anxiety, vulnerability, and depression.

Methods Employed: Structured and open-ended interviews. A stratified random sample of 1910 children from the third through the twelfth grades in a large urban school system was interviewed. Additional brief interviews were conducted with 400 parents to obtain certain essential background information.

Major Findings: (1) Contextual dissonance--Contextual dissonance or consonance refers to the discrepancy between, or concordance of,

the individual's social characteristics and those of the population by which he is surrounded. In this view, the environment is not in itself consonant or dissonant; it is only consonant for a given individual with reference to a particular characteristic. Although social science has neglected this topic, it appears to be of obvious relevance for the self-concept. These data and data collected earlier by the Principal Investigator on a sample of adolescents were analyzed with reference to three dimensions of the self-concept: self-esteem, stability of the self-concept, and group identification.

Identity contexts--An individual's social identity consists of the groups, statuses, or social categories by which he is socially recognized and classified in the society: race, religion, sex, national origin, age, class, etc. Looking first at the religious context (pupil's religion and neighborhood religion), the data indicated that religious dissonance was associated with lower self-esteem and higher rates of psychophysiological indicators of anxiety. For example, Jewish children raised in predominantly gentile neighborhoods were found to have somewhat lower self-esteem and more psychosomatic evidence of anxiety than Jewish children raised in Jewish neighborhoods. Similarly, Protestants raised in Catholic or Jewish neighborhoods had somewhat lower self-regard than those raised in neighborhoods inhabited chiefly by co-religionists; and the same held true for Catholics. In general, the relationship between the characteristics of an individual and those of his immediate environment have an important bearing upon his feeling of self-worth.

Examination of the racial context yielded similar results. Black children in dissonant contexts (predominantly white schools) scored lower on a self-esteem measure than black children in consonant contexts (predominantly black schools). In part, this turned out to result from the obvious fact that the former were more likely than the latter to have experienced direct racial discrimination.

Social class dissonance has been difficult to study because economic segregation is very widespread and is further contaminated with race segregation. Nevertheless, it is possible to classify the socioeconomic status (Hollingshead scale) of the child and the mean SES level of his school. Considering black and white children separately, the data showed that both black and white children whose SES was below that of their schools tend to have somewhat lower self-esteem; among white children, interestingly, those whose SES was above average also had lower self-esteem, but this was unclear among higher class black children.

Religion, race, and class are, of course, socially ranked elements of identity. Whether similar effects would appear with regard to social categories which are not socially ranked, or in

environments in which minority group members satisfy some special need or fulfill a special function, remains to be learned.

Competence contexts--In addition to the groups, statuses, or social categories which constitute the individual's social identity, he is endowed with a multiplicity of traits. Insofar as these traits represent socially evaluated characteristics, their sociological relevance for self-esteem is obvious. In the Baltimore study, it was found that black borderline pupils attending predominantly black schools where the average marks were relatively high had lower self-esteem than those in schools where the average level of performance was closer to their own. Furthermore, some psychologists have reported that well-functioning retardates may suffer self-esteem disturbances when transferred to classes of normals. They have shifted from a context in which they compare favorably to one in which they do not.

Values contexts--Systematic research in this area is almost non-existent. Here consonance is not reflected in the concordance of individual and environmental characteristics, but in the relation of the individual characteristic to the values predominant in that environment. In high schools where athletic ability was highly valued, boys designated as "best athlete" appeared to have higher self-esteem than boys of equal athletic skill in schools where this quality was less highly regarded.

Analysis of our data thus indicates that identity contexts, competence contexts, and values contexts may all affect the individual's self-esteem. There is some evidence to suggest that it may also affect the stability of the self-concept. Since the individual's self-concept is his basic cognitive apparatus for structuring the world, an unstable self-concept is highly anxiety-provoking; our data show a strong relationship between self-concept stability and psychophysiological indicators of anxiety.

The study found that, controlling on self-esteem, black children at each school level in dissonant racial contexts were conspicuously more likely than those in consonant contexts to have unstable self-concepts. With regard to religious dissonance (again controlling on self-esteem), no association between contextual dissonance and instability appeared among Jews (actually there was a relationship but it was entirely due to self-esteem), but some association appeared among Protestants, and a definite relationship among Catholics. While it is not entirely clear how contextual dissonance exercises its effect, there is reason to think that the attitudes of members of another group toward the individual may not correspond with his own: in failing to provide interpersonal confirmation of his self-hypothesis, these reflected appraisals are likely to give rise to uncertainty and instability in his self-image.

Finally, we considered whether contextual dissonance might affect group identification and group pride. Our study contained six items which appeared to reflect racial group identification. The results showed that in junior high schools, the context appeared to have no effect on racial identification, but, in the elementary and senior high schools, contextual dissonance was associated with somewhat weaker group identification. In general, however, there was very little evidence of the strong racial group dis-identification postulated by so many writers.

That the social environment is an important factor in personality development and social behavior is both a matter of immediate experience and an established dictum of social psychology. Yet the same social environment will affect different people differently. One reason, this research suggests, is that this environment may be consonant for some, dissonant for others. The present research has sought to specify how different contexts may affect different aspects of the self-concept.

(2) Group Disidentification--That the individual's membership and reference groups may constitute important components of the self-concept has long been recognized in social psychology. It is thus understandable that writers on minority group problems have used such expressions as "self-hatred among Jews" or "black self-hatred" to describe the plight of minority group members who internalize the majority attitudes toward themselves. What such discussions overlook, however, is that attitudes toward one's group and attitudes toward oneself are entirely different. Our analysis has therefore attempted to examine the empirical question: Does group disidentification lead to low self-esteem?

In this study group identification was examined in terms of desire to be a group member, pride in the group, importance of the group, and inseparability of group and self. The results show virtually no relation between group identification and individual self-esteem. The self-esteem of black children who did not identify with their groups was just as high as that of black children who identified strongly. Religious group identification yielded essentially similar results.

Further data analysis was undertaken to account for this unexpected empirical finding. Part of the reason, it turns out, is that the group is a molecular element of the self-concept while global self-esteem is a molar judgment based on a complex synthesis of elements. Thus, we sought to determine how much the child identified with 16 ego-extensions (e.g., mother, father, family, siblings, race, religion, clothes, school work, friends, father's job, school). The results show that while race and religion are widely felt to be an integral part of the self, mother, father,

and siblings are even more generally accepted as ego-extensions, and other things, such as clothes, friends, father's job, and school, are also chosen by more than half the sample. What writers on minority groups consider of prime, if not exclusive, importance must in reality take its place alongside many others important to the individual in forming his general self-assessment.

What holds true for group memberships obtains equally with regard to personal attributes. There are many personal attributes which may arouse pride or shame in the individual and which may be experienced as more central to the self than one's racial or religious group. One way to judge this matter is to see whether the individual's pride or shame in certain traits (intelligence, appearance, social skill) are more closely related to his global self-esteem than certain aspects of social identity (race or religion). The data show that the trait-self-esteem correlations are much stronger than the group-self-esteem correlations. Furthermore, those children who are proud of their traits but not proud of their groups have higher self-esteem than those who are proud of their groups but not proud of their traits.

One additional point frequently overlooked is that race and religion are ascribed statuses, i.e., assigned the child at birth, and have nothing to do with the individual's own achievement. Hence, we hypothesized that feelings of pride or shame in these groups might have less bearing upon the general feeling of self-worth than statuses for which the individual was himself responsible. The data turned out to be consistent with this reasoning: the relationship between group pride and self-esteem was much weaker than the relationship between school marks or social success and self-esteem.

Whether the individual is proud or not proud of his racial or religious group, then, has little bearing upon his global feeling of self-worth. While the individual's race and religion are important, they are only two of a large number of elements that enter into self-esteem formation; nor are they necessarily experienced as central to the self. In those cases in which these groups <u>are</u> felt as integral to the self, however, the data show that lack of pride in them <u>does</u> lower self-esteem.

<u>Scientific Significance</u>: Problems of the self-image are often involved in mental health and illness. Yet little is currently known about how position in the social structure, and the characteristic experiences associated with this position, influence the formation of the self-image. This study is designed to contribute knowledge to this area, and to learn how the self-image changes with age.

Proposed Course of Project: Data analysis will continue through the coming year, at the University of Buffalo, where the Principal Investigator has accepted an appointment.

In particular, we intend to be looking at committed and idealized images of children in order to learn how their location in the social structure affects the self-fantasies they construct as well as their realistic aspirations. In contrast to other studies, our focus will be more heavily on the "sociological" aspects of the self-concept, such as "style of life" (how the individual would like to pursue his future round of life), social statuses (occupation, group memberships), social and material environment (house, neighborhood), etc. To our knowledge, virtually no studies have examined the significance of the child's location in the social structure for the self-conceptions and self-fantasies that emerge within that particular environmental context.

Honors and Awards: None

Publications:

Rosenberg, M.: Which significant others? American Behavioral Scientist, 6: 829-860, 1973.

Rosenberg, M.: The logic of suppressor variables. Public Opinion Quarterly, 37: 359-372, 1973.

Simmons, R. G., Rosenberg, F., and Rosenberg, M.: Disturbance in the self-image at adolescence. American Sociological Review 38: 553-568, 1973.

Serial No. M-S-SP-4
1. Socio-environmental Studies
2. Section on Social Process
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Individual Differences in Survival and Reproduction Among Old Colony Mennonites in Mexico

Previous Serial Number: Same

Principal Investigators: Gordon Allen and Calvin Redekop (non-PHS)

Other Investigators: None

Cooperating Units: Goshen College, Goshen, Indiana

Person Years

Total:	1-1/6
Professional:	1
Other:	1/6

Project Description:

Objectives: To demonstrate the presence or apparent absence of selective survival and reproduction with respect to individual behavioral characteristics. Also to study natural patterns of marriage and reproduction in a relatively simple agricultural society of traditional European type.

Methods Employed: The population under study consists of about 13,000 German-speaking Mennonites in a politically integrated cluster of 46 villages in northern Mexico. These people were selected because of their simple and uniform culture insulated from mass communication media and because of their apparently unrestricted fertility. Also important was the fact that, unlike some similar religious isolates, this society supports a considerable degree of economic competition.

Information was gathered by means of a combined census and survey that reached a third of the population. This has been supplemented with photographs of all vital records kept by the church, a public list of farmers and their holdings, a list of all village and church leaders in the 40-year history of the population, and records of Mennonite births in local hospitals.

Editing of the data for accuracy and consistency was the most time-consuming part of the project, because each set of records, even our own census, was collected and recorded by poorly educated farmers. After editing, the main data sets were subjected to cross-tabulation for general descriptive purposes. The survey data and the public list of farmers are now being used in correlation analyses that will yield ratings for each farmer in several dimensions of socio-economic status. These ratings, together with the vital statistics obtained in the census, will be linked to the church records.

The final phase of the analysis will consist of a search for relations between past vital events and socio-economic variables.

Major Findings: Several indicators of material wealth show that there is very substantial and significant variation among the villages in this dimension. For example, one village has no farm with over 20 cows or over 100 acres; in another village 70% of farmers have over 20 cows and 35% own more than 100 acres. Thus village of residence is almost certainly a status symbol, and this "simple agricultural" society exhibits a rather high degree of economic segregation.

Scientific Significance: Natural selection is apparently the sole agent responsible for the progressive evolution and maintenance of human mental capacities. The study of selection in a pre-industrial European society may indicate some of the influences that have produced present genetic tendencies and draw a base line for future studies of selection under modern conditions.

Proposed Course of Project: Analysis of the data is expected to occupy between two and three years.

Honors and Awards: None

Publications:

Allen, G. and Pettigrew, K. D.: Heritability of IQ by social class: evidence inconclusive. Science, 182: 1042-1044, 1973.

Serial No. M-S-SP-5
1. Socio-environmental Studies
2. Section on Social Process
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Social Origins of Stress

Previous Serial Number: Same

Principal Investigator: Leonard I. Pearlin

Cooperating Units: None

Person Years

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: This study examines the interrelations between the recurring problems and strains that people experience in their everyday lives, their methods of dealing with such experiences, and the emotional stress that may result. Much of the inquiry is organized around the strains that occur in the major role areas of work, economic maintenance, parenthood, and marriage. People of different socio-economic classes, races, sex and age ranks will be compared both with regard to the strains they experience in these institutional roles and the techniques they employ in coping with them. In this way it will be possible to understand better the relationships between elements of social structure, enduring strains, coping repertoires, and states of stress.

Methods Employed: The data have been gathered from a cluster sample of 2300 households in the Chicago urbanized area. An adult member from each household was interviewed with a standardized schedule developed over a two-year period.

Major Findings: Much of the recent work has been aimed at marriage relations. The social statuses that people bring to marriage have an important part in the strains and stresses that appear in this domain. In marriages between people of unequal status, the person of the higher origins is relatively likely to experience difficulties in several crucial areas, such as the ability to communicate meaningfully with his spouse, to give and receive affection, and to share important values. The mate who bears the higher status

is also disposed to feel that in the overall give and take of his marriage, he emerges at the losing end of the exchange. By contrast, people who have married up are least likely to experience these strains. Where such strains do exist in marriage, they are likely to be closely associated with stress. Thus the way that social status is combined in marriage affects key elements in marital relations and where these elements are disrupted, emotional stress is likely to ensue.

Scientific Significance: This analysis focuses on interpersonal relations within the family, an institution widely recognized as vitally important to the emotional well-being of people. In looking at these relations, social structural factors and enduring strains have been emphasized instead of the transitional crises and individual pathologies that are typically examined in studies of the family's contribution to individual stress. The emphasis employed here begins to reveal the complex interconnections between social structural conditions, the organization of interpersonal relations, recurrent conflicts, and emotional distress.

Proposed Course of Project: The above analysis will be completed and submitted for publication. Work has already begun that is directed at differences in the strains and stresses experienced by men and women, and this will be continued.

Honors and Awards: None

Publications: None

Serial No. M-S-P(C)-23
1. Socio-environmental Studies
2. Personality and Environment
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cultural and Psychodynamic Factors in the Occurrence and Treatment of Psychiatric Illnesses in Japan, Taiwan, and the United States

Previous Serial Number: Same

Principal Investigator: Carmi Schooler

Other Investigators: Hsien Rin

Cooperating Units: Department of Psychiatry, National Taiwan University, Taipei, Taiwan, Republic of China

Person Years

Total:	5/12
Professional:	1/12
Other:	4/12

Project Description:

Objectives: (1) To identify and describe the types and symptom patterns of psychiatric illnesses in Japan and Taiwan. (2) To determine the relationship of these types of illnesses to social, cultural, and psychological variables in Japanese and Chinese cultures. (3) To compare the results obtained with results from similar data on psychiatric patients in the United States.

Methods Employed: Symptom data discussed in the present annual report were gathered by having doctors in each hospital in Japan and Taiwan fill out a research instrument for each patient which obtained relevant background and family information and a semi-structured narrative description of the course of the patient's illness. The narrative description was then coded for the presence or absence of 90 symptoms; the set of symptoms represents an extended version of the symptom checklist used in an earlier study. All symptoms which occur in more than 7-1/2% of the combined Japanese and Taiwan-Chinese population are included in a series of principal components factor analyses that are rotated to simple structure. The subjects in the two samples consist of (1) all admissions to five psychiatric hospitals in Tokyo during a twelve-month period in 1963-64 for a

total of 994 patients; (2) all admissions to three psychiatric hospitals in Taipei during a two-year period, 1967-68, for a total of 1,009 patients.

Major Findings: The major findings of this study were reported last year, although some additional analyses were done this year. These more recent analyses focused on two areas: those in which the major independent variable studied was diagnosis, and those which looked directly at the effects of cultural factors not previously analyzed. The diagnostic analyses examined the criteria used to classify patients into the schizophrenic sub-types. The comparison of Taiwan-Chinese and Japanese schizophrenics indicated that similar sub-typing principles were used in both countries, while the analysis of data from within the Republic of China revealed that diagnostic sub-typing of schizophrenics in that country seemed to follow the general patterns of the American Psychiatric Association diagnostic manual.

The further analyses of cultural factors had several foci. One involved the comparison of the results of the Lorr IMPS scores of the Taiwan-Chinese schizophrenics vs. the American schizophrenics in the 8-hospital collaborative study of the effects of phenothiazines on acute schizophrenics. The basic finding is that, with the exception of disorientation, the American patients are sicker on all of the symptom factors. The absence of any more interesting pattern in the results is puzzling.

A search was also made for cross-ethnic differences within Taiwan. A relatively large number of analyses were done but only one significant main effect difference between the Mainland- and Taiwan-descended Chinese was found. Furthermore, when the interactions in symptomatology between ethnic background and age were examined, about as many significances as would be expected by chance were found. Thus, it would seem that once social class and rurality are controlled, no reasonable case could be made for any overall or age-specific differences between the two major ethnic groups in Taiwan in the type of symptomatology they display.

The two Chinese ethnic groups do differ in the content of delusions they have. Examining all those delusions held by more than 5% of the total sample, we found that mainlanders show more delusions of reference; the Taiwan-descended Chinese show more delusions of persecution, grandeur, and sexual delusions. This is particularly true for females at all age levels. Similarly, we found that, independent of culture, age differences in types of delusions exist among women, but not among men. Delusions of reference and control decline with age, while persecution delusions increase with age and sexual delusions peak at ages 30-39. Social class does not have much effect on the nature of delusions.

An index of traditionality was created based upon the characteristics of the individual's family. We looked at its effects, both in Taiwan and in Japan. Looking at the combined factor scores, it appears that in Taiwan those from traditional backgrounds are more apathetic and hebephrenic and less hostile; while in Japan the only significant difference is that those from traditional backgrounds are more apathetic.

Scientific Significance: This year's analyses were intended to fill gaps left in the analyses whose results were reported in earlier annual reports. The lack of powerful and consistent findings in this year's analysis suggests that the earlier work did uncover the most important findings in the data. The first of these previously reported findings is that factor analysis of the symptom patterns of hospitalized Taiwan Chinese and Japanese mental patients reveals both cross-culturally applicable and culturally specific symptom factors. The second major finding is the power and clarity of the cross-cultural differences in psychopathology which emerge. While the Japanese are likely to have disorders in arousal levels, to turn against themselves and to see the major source of their problems as being within themselves, the Taiwan Chinese are much more likely to direct their symptomatology outward, to act out against others, and to perceive the outside world in unreal ways. The third major finding is that women in each culture are more likely than men both to show more severe symptomatology and to have a higher incidence of the symptom patterns which reflect the particular symptomatology of their culture.

Proposed Course of Project: The results of this year's analyses have been sent to the Taiwanese collaborator who may integrate them for publication in a scientific journal.

Honors and Awards: None

Publications:

Rin, H., Schooler, C. and Caudill, W. A.: Culture, social structure and psychopathology in Taiwan and Japan. Journal of Nervous and Mental Disease, 157: 296-312, 1974.

Serial No. M-S-P(C)-27
1. Socio-environmental Studies
2. Personality and Environment
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Parental Care and Child Behavior in Japan and the United States

Previous Serial Number: Same

Principal Investigator: Carmi Schooler

Other Investigators: None

Cooperating Units: None

Person Years

Total:	1
Professional:	1/6
Other:	5/6

Project Description:

Objectives: (1) To identify and describe patterns of parental care and child behavior in Japanese and American families. (2) To determine the relationship of these patterns of behavior to personality development, psychopathology, culture, and social structure in the two countries.

Methods Employed: The basic design of the study and methods used have been described in detail in previous reports. Briefly, this is a longitudinal observational study of the behavior of parents and children in the home over the first six years of life in Japan and the United States. During 1961-64, 30 Japanese and 30 American middle-class mother-infant pairs were selected for observation. The infants were first-born, normal babies, and were three-to-four months of age at the time of observation. The first 20 Japanese and the first 20 American cases were selected for follow-up study at 2-1/2 years of age, and observations in the home were completed during 1963-1967. These same children were studied for a third and final time at 6 years of age during 1967-70, and all observations have been completed.

Major Findings: This past year has been spent in carrying out analyses of the data gathered when the subjects were 2-1/2 and 6 years of age. The results indicate that the essential cross-cultural

differences in behavior found when the subjects were infants are repeated at age 2-1/2 and again at age 6. Compared to Japanese, Americans are more active, more vocally and physically emotional, more independent, and more likely to manipulate functionally both their social and physical environments. At each of the childhood periods the cross-cultural differences in the behavior of parents and children seem to mirror each other, and to be logical outcomes of the cross culturally different patterns of parent-child interaction at infancy.

During infancy the American mother views her baby, at least potentially, as a separate and autonomous being who should learn to do and think for himself. For her, the baby is from birth a distinct personality with his own needs and desires that she learns to recognize and care for. She helps him to learn how to express these needs and desires through her emphasis on vocal communication so that he can "tell" her what he wants and she can respond appropriately. Thus in America even the period of infancy is characterized by an emphasis on the psychological separateness of mother and infant, and the development of relatively sharp ego boundaries between the two. The American mother de-emphasizes the importance of physical contact such as carrying and rocking, and encourages her infant through the use of her voice to explore, and learn to deal with, his environment by himself. The American mother does more encouraging of her baby to reach out for toys, and to be more physically active. Just as the American mother thinks of her infant as a separate individual, so also does she think of herself as a separate person with her own needs and desires, which include time apart from her baby to pursue her own interests, and to be a wife to her husband as well as a mother to her baby.

In Japan, in contrast to America, the mother views her baby much more as an extension of herself, and psychologically the boundaries between them are blurred. Because of the great emphasis in Japan on the close attachment between mother and child, the mother is likely to feel that she knows what is best for her baby, and there is no particular need for him to tell her verbally what he wants because, after all, they are virtually one. Given this orientation, the Japanese mother places less importance on vocal communication and more on physical contact; also, for her there is no need for hurry as the expectation is that she will devote herself to her child without any great concern for a time away from him even for a separate time to be with her husband. This expectation extends throughout the night because of the Japanese custom of parents and children sleeping together in the same room. From these results of the infant study it seems likely that a considerable amount of culturally patterned behavior has already come into being by 3 to 4 months of age. This year's analysis of data from later time periods strongly suggests that this did indeed happen.

Some caution is needed in generalizing from these findings. The sample consists of individuals born at a given point in time (1961-1964), into a given social class (middle), and in two specific countries (Japan and the United States). It's quite possible that results would have been different if the subjects from the two countries had been born at another time, or into a different social class. An even greater limitation is that we are comparing only two cultures; thus, to say that Japanese children are less verbal or less independent than American children does not imply that they are non-verbal or dependent in any absolute sense. In fact, at age 6 Japanese children show verbal behavior in 60 percent of the observations. It is quite possible that, if Japanese or American children had been compared to children in some other culture, the tenor of these findings would be different.

Scientific Significance: Even with many analyses remaining yet to do, Caudill's achievement is clear. With a sample of only 20 American and 20 Japanese children he was able to demonstrate conclusively meaningful and consistent cross-cultural differences in the behavior of children and their caretakers which persist from infancy through early childhood. Since the cross-cultural differences in child behavior he found seem to parallel many of those which distinguish Japanese from American adults, and since he was able to show that the genetic contribution to these differences is at most minimal, Caudill's work marks an important step forward in demonstrating the early and persistent effects of culture on personality.

Proposed Course of Project: At present our analyses are far from complete. Many other independent variables remain to be investigated. In the analyses of the 2-1/2 and 6-year data we have collapsed the behavior of all of each child's caretakers, disregarded possible effects of different locations in which behaviors occurred, and made no attempt to explore sequential behavior patterns. Most importantly, we have not taken into account all the other information we have available to us in histories, interviews, and observer's notes which could be used to characterize the personal characteristics of the children and their principal caretakers. We are presently attempting to pursue many of the above paths. When these analyses will be completed, the results will be presented to a scientific journal for publication.

Honors and Awards: None

Publications:

Caudill, W. A. and Schooler, C.: Child behavior and child rearing in Japan and the United States: an interim report. Journal of Nervous and Mental Disease, 157: 323-338, 1974.

Caudill, W. A.: The influence of social structure and culture on human behavior in modern Japan. Journal of Nervous and Mental Disease, 157: 240-257, 1974.

Caudill, W. A. and Frost, L.: A comparison of maternal care and infant behavior in Japanese-American, American, and Japanese families. In Lebra, W. P. (ed.): Youth, Socialization, and Mental Health: Volume III of Mental Health Research in Asia and the Pacific. Honolulu, The University Press of Hawaii, 1973.

Caudill, W.: Tiny dramas: vocal communication between mother and infant in Japanese and American families. In Lebra, W. P. (ed.): Transcultural Research in Mental Health: Volume II of Mental Health Research in Asia and the Pacific. Honolulu, The University Press of Hawaii, 1972.

Serial No. M-S-P(C)-38
1. Socio-environmental Studies
2. Personality and Environment
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Interrelationships between Social Interaction, Psychological Functions, Perceptual Style, Physiological Arousal and Personal History Factors Among Schizophrenics and Normals

Previous Serial Number: Same

Principal Investigator: Carmi Schooler

Other Investigators: Theodore Zahn and Monte S. Buchsbaum

Cooperating Units: Laboratory of Psychology, NIMH; Saint Elizabeths Hospital; DCRT

Person Years

Total:	1
Professional:	1/6
Other:	5/6

Project Description:

Objectives: The objective of this series of studies is to gain understanding of the schizophrenic process by examining the complex interactions between the individual's psychological and physiological systems and between both of these and his social environment. This is done by examining the interrelationships between task performance, physiological arousal, perceptual style, personality characteristics, and everyday behavior patterns in schizophrenics and normals.

Methods Employed: Several approaches are presently being followed. (1) The level of physiological arousal and task performance under different social conditions of both schizophrenics and nonschizophrenics have been measured and related to variations in perceptual style and personal history. (2) Data has been gathered for a study which involves the interrelating of measures of evoked response, perceptual style, skin temperature (a measure of physiological arousal) and symptomatology. (3) In order to facilitate the continuance of both of the above types of study, a generalized computer program has been developed to simplify the analysis of measure of physiological arousal and to relate these measures to each other and to other

aspects of the experimental situations. (4) A study is being developed which will examine the exact nature of psychophysiological arousal during the different stages of perceptual task performance. In its initial stages this study will be carried out with normal persons and if the results seem promising it will be continued with schizophrenics.

Major Findings: Although there are no new substantive findings, this year has seen the completion, except for a final check-out, of the generalized psychophysiological computer program, the completion of data collection and analysis of the evoked response portion of the data in the study of the interrelationship of symptomatology and psychophysiology, and the near completion of the developmental phase of the study of the pattern of physiological arousal during perceptual tasks among normals.

Scientific Significance: The senior investigator became interested in the present line of investigation as a result of his earlier studies of the social psychology of schizophrenia. The findings of interactions between the social situation, the schizophrenic's functioning, and his physiological reactivity pointed to a general weakness in the way this earlier series of studies had been carried out. It had been conducted primarily from one research perspective and was concerned with only a limited area of the individual's functioning. Although the findings were significant they had not been very powerful, possibly because differences in the nature of physiological reactivity of different subgroups of schizophrenics may have produced contrasting, or at least differing, results.

A review of the literature revealed that the general concentration on one aspect of the individual which had marked these social psychologically oriented studies was at least as pronounced in studies in other areas. It was therefore not at all surprising that the weak results which marked the series of social psychological studies were at least as powerful as the results of studies approaching the problem of schizophrenia from other directions. Thus, for example, years of search for biochemical differences between schizophrenics and others have resulted in no reliably replicable findings. The effects of genetics appear to be less and less powerful, the better controlled the study. Putative social class differences between schizophrenics and others do not seem to exist under all conditions and where they do exist it is far from clear whether such differences are related to the cause of the disease or its effects. Those theoretically based systems of classifying patients into subgroups which have been investigated have not produced consistent findings and, in fact, the most highly touted mode of classification--pre-morbid adjustment--proved totally unable to pass the test of controlled replication.

The lack of findings has certainly not been due to any lack of theories or hypotheses. Hypotheses have been brought forth with the ease of the proverbial blind men describing the elephant, e.g., those studying tails describing the elephant as long, thin, and floppy; those studying problem-solving seeing the essence of schizophrenia in thought disorder.

Two courses seem to be presently open. One can keep on making hypotheses from limited bodies of data, hoping to achieve the flash of insight which will permit us to see from our limited point of view what the elephant is really like, or one can go ahead with the duller task of trying to do studies which attempt to coordinate data from many vantage points in order to get enough information to make meaningful hypotheses possible. The present series of studies represents an attempt to follow the second of these courses with the aid of modern multivariate techniques and computer technology.

Proposed Course of Project: Data collection will be continued and analyses of already collected data will be continued and the results prepared for publication in a scientific journal.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-1

1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neural substrate of mirror display in squirrel monkey (Saimiri sciureus).

Previous Serial Number: Same

Principal Investigators: Paul D. MacLean

Other Investigators: Robert E. Gelhard, Thalia D. Klosteridis, Levi Waters

Cooperating Units: None

Man Years

Total:	1.7
Professional:	0.5
Other:	1.2

Project Description:

Objectives: This long-term study utilizes the innate display behavior of the squirrel monkey (1) for obtaining information about the cerebral representation of species-typical behavior and (2) identifying structures involved in the integration of autonomic and somatic functions in visually guided sociosexual behavior. Last year's report described the loss of display behavior following lesions of the globus pallidus. The present experiments assess the effects of lesions of major efferent pathways from the medial globus pallidus.

Methods Employed: The mirror display test for male gothic squirrel monkeys has been described in previous project reports (M-NP-LI-17, 1963 and 1964). After animals reach criterion in 30 or more trials, small electrocoagulations are placed at the point of confluence of the ventral and dorsal divisions of the ansa lenticularis. The monkeys are then retested for several weeks.

Major Findings: Bilateral lesions of the ansa lenticularis were placed in three monkeys identified as N-4, O-4, and P-4. For comparison, lesions were aimed at the medial forebrain bundle in M-4. In O-4, P-4, and M-4, the lesions were remarkably symmetrical and well placed. Additional structures involved in each animal were as follows:

N-4 Bilateral interruption of mammillothalamic tract (MT); ventral parts n. ventralis anterior and lateralis; medial parts of zona incerta and H1 and H2.

O-4 Bilateral MT.

P-4 Bilateral MT and fornix.

M-4 Right ventral division of ansa; bilateral fornix at mid-hypothalamic levels.

N-4 developed moderate major motor deficits and never again showed interest in the mirror. After 30 days O-4 recovered the capacity for a full display, but then from 94-125 days gave only five abortive displays, with only partial erection and no somatic components except vocalization. P-4 appeared entirely normal the day following operation, but never again showed any interest in the mirror during 71 days of follow-up. M-4 also showed no motor deficit. During the 70-day follow-up, there were a few sporadic displays with all somatic components present but in no instance full erection. This latter finding was not unexpected since it had been previously shown by stimulation studies in this laboratory that the medial forebrain bundle is a major effector pathway for penile erection.

Significance to Biomedical Research and Program of the Institute. Previous work of this project showed that after extensive destruction of pathways to and from the striatal complex and limbic system, monkeys may recover spontaneous movement and the ability to feed themselves. Despite the intact great pathways to and from the neocortex, however, these animals no longer showed their characteristic displays or other typical forms of simian behavior. In a word, they no longer behaved like squirrel monkeys. In other experiments it had been found that bilateral interruption of individual limbic pathways such as the fornix or mammillothalamic tract did not alter the expression of display behavior. Significantly, the present experiments have shown that additional bilateral lesions involving major projection pathways of the globus pallidus virtually eliminate the display. The findings therefore help to pinpoint pathways to and from the reptilian and paleomammalian counterparts of the primate forebrain

that are essential for highly organized forms of species-typical behavior. Viewed clinically in the light of various case material, the accumulated results indicate that the pathways in question are fundamental for the expression of non-verbal behavior.

Proposed Course of the Project: To be continued.

Honors and Awards: Items pertaining to Dr. MacLean are as follows:

1) Lecture: "Neuroethological Findings on the Striatal Complex," Department of Pharmacology, Howard University Medical School, Washington, D.C., October 31, 1973.

2) Invited Chairman and Speaker: Symposium on "Brain Mechanisms of Social Behavior," Meetings of the Society for Neuroscience, San Diego, California, November 8, 1973.

3) Plenary Lecturer: "Psychoanalyzing the Triune Brain," The American Psychoanalytic Association, Waldorf-Astoria Hotel, New York, New York, December 14, 1973.

4) Invited Lecturer: "Brain Mechanisms of Species-Typical Behavior," Department of Psychiatry, Washington University School of Medicine, Barnes & Renard Hospitals, St. Louis, Missouri, March 6, 1974.

5) Invited Speaker: "An Evolutionary Approach to Brain Research on 'Prosematic' (non-verbal) Behavior," The Daniel S. Lehrman Memorial Symposium on Reproductive Behavior and Evolution, Institute of Animal Behavior, Rutgers University, April 27, 1974.

6) Invited Speaker: "Brain Mechanisms of Sexual and Related Behaviours," The First International Symposium on the Pharmacology of Sexual Behaviour, The University of Cagliari, Forte Village, Sardinia, June 3-7, 1974.

7) Invitation by The Honorable Caspar W. Weinberger, Secretary of HEW, to present the work of the Laboratory at the HEW Staff Conference, Wednesday, February 13, 1974.

Publications:

MacLean, P.D.: An evolutionary approach to the investigation of psychoneuroendocrine functions. In Lissak, K. (ed.): Hormones and Brain Functions. The Proceedings of the Second Congress of the International Society of Psychoneuroendocrinology. New York, Plenum Publishing Corp., 1973, pp. 379-389.

MacLean, P.D.: A triune concept of the brain and behavior: Lecture I. Man's reptilian and limbic inheritance: Lecture II. Man's limbic brain and the psychoses; Lecture III. New trends in man's evolution. In Boag, T. and Campbell, D. (eds.): The Hincks Memorial Lectures. Toronto, University of Toronto Press, 1973, pp. 6-66.

MacLean, P.D.: The brain's generation gap: Some human implications. Zygon J. of Relig. and Sci., 8: 113-127,1973.

MacLean, P.D.: Influence of limbic cortex on hypothalamus: New anatomic and microelectrode findings. In Lederis, K. and Cooper, K.E. (eds.): Recent Studies in Hypothalamic Function. Basel, S. Karger AG, 1974, pp. 216-231.

MacLean, P.D.: Evolutionary trends of the triune brain: From imitation to creativity. In Royaumont Symposium on "The Unity of Man" (in press). Full length version to appear in Sesquicentennial Symposium "On Human Adaptation, Past and Future," Institute of Living, Hartford, Conn.

MacLean, P.D.: Brain mechanisms of elemental sexual functions. In Freedman, A.M., Kaplan, H.I. and Sadock,B.J. (eds.): Comprehensive Textbook of Psychiatry. Baltimore, The Williams and Wilkins Company, (in press).

MacLean, P.D.: Sensory and perceptive factors in emotional functions of the triune brain. Proceedings of the Karolinska Symposium on "Parameters of Emotion" (in press).

MacLean, P.D.: Hippocampal influence on hypothalamus: Recent microelectrode and anatomic findings. In Isaacson, R.L. and Pribram, K.H. (eds.): The Hippocampus: A Comprehensive Treatise. New York, Plenum Publishing Corp., (in press).

Ng, L.K.Y., Gelhard, R.E., Chase, T.N., and MacLean, P.D.: Drug-induced dyskinesia in monkeys: A pharmacologic model employing 6-hydroxydopamine. In Barbeau, A., Chase, T.N., and Paulson, G.W. (eds.): Huntington's Chorea. New York, Raven Press, 1973, pp. 651-655.

Serial No. M-LBEB-CN-2
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Unit study of interoceptive inputs to the cingulate cortex of squirrel monkey.

Previous Serial Number: Same

Principal Investigators: Robert A. Hallowitz and Paul D. MacLean

Other Investigators: Patrick J. Carr and Thalia D. Klosteridis

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description:

Objectives: There is little information regarding the important question of visceral influences on the forebrain. The present study tests the effects of vagal volleys on unit activity of the cingulate and supracingulate cortex. The cingulate gyrus which is a major part of the limbic system is known to affect respiratory, cardiovascular, and genital functions.

Methods Employed: Single unit activity is recorded with an improved theta-glass micropipette (5-10 MOhms) in awake, sitting squirrel monkeys prepared with a chronically fixed stereotaxic platform. Bipolar, concentric, stainless steel electrodes are chronically implanted in the jugular foramen next to the vagus and are led to a plug on the platform. As a control, somatic stimulation is applied through electrodes in the neck.

Major Findings: More than 1000 units located between frontal planes F4 and F20 have now been tested in 11 animals.

Of 518 cingulate units 101 (19.5%) were responsive to

vagal volleys, with 36 showing initial excitation at regular latencies, 51 showing inhibition, and 14 an increase in firing rate.

In supracingulate cortex 110 (22%) of 498 units were responsive, with 36 showing initial excitation, 28 initial inhibition, and 46 an increase in firing rate.

The middle cingulate area had a higher percentage (33%) of responsive units than the rostral and caudal areas.

Cingulate units responded at significantly shorter latencies (8-20 msec for the majority) than supracingulate neurons, suggesting their activation by more direct vagal pathways (see Project No M-LBEB-CN-15).

Significance to Biomedical Research and Program of the Institute. Although visceral symptoms are a prominent feature in psychoneurotic, psychosomatic, and psychotic illnesses, little has been learned about brain mechanisms underlying the subjective manifestations. Visceral symptoms are a dreaded part of the withdrawal manifestations in narcotic addiction, adding to the difficulties of enlisting the cooperation of patients in submitting to treatment. The present study together with Project No. M-LBEB-CN-15, contributes to the knowledge of sensory mechanisms affecting cortical participation in visceral activity.

Proposed Course of the Project: To be terminated with the publication of the results.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-8
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neural substrate of olfaction in the rat.

Previous Serial Number: Same

Principal Investigator: Burton M. Slotnick

Other Investigators: Lois A. Gutman and Thalia D. Klosteridis

Cooperating Units: None

Man Years

Total:	1.7
Professional:	0.3
Other:	1.4

Project Description:

Objectives: These experiments utilize an operant conditioning procedure for analyzing the effects of basal rhinencephalic lesions on detection and discrimination of odors.

Methods Employed: Prior to surgery rats were trained in a wind-tunnel olfactometer (see previous report, 1971) to signal detection of a 1-sec odor sample of dilute amyl acetate. After extensive overtraining, lesions were placed in the anterior limbs of the anterior commissure (2 rats), the rostral portion of the lateral olfactory tract (2 rats), in the olfactory tubercle (2 rats), and in the amygdala (1 rat). After postoperative tests for retention, animals were trained on a series of 2-odor discriminations of increasing difficulty. Upon completion of training the two animals with transection of the lateral olfactory tract (LOT) were subjected to lesions in the olfactory tubercle and retested.

Major Findings: One rat with an anterior LOT transection showed a marked deficit in retention of the detection task and little or no ability to solve 2-odor discriminations. In the second rat the LOT was transected more posteriorly and it had only a

moderate deficit in retention, solving all but the most difficult of the 2-odor tasks. When retested after lesions destroying the medial and lateral olfactory tubercle, both showed deterioration of performance but were still able to detect odors and tested significantly better than chance on some simple 2-odor tasks. The two rats which received olfactory tubercle lesions only had no deficits in retention or subsequent learning of new odor discriminations.

No deficits in retention or subsequent learning were observed in animals with transection of the anterior limbs of the anterior commissure or with large lesions destroying the amygdala.

Significance to Biomedical Research and Program of the Institute. These accumulated studies represent the only systematic investigation of bulbofugal pathways in olfactory discrimination. Our results suggest that this afferent system is more extensive than hitherto indicated by neuroanatomical studies. No one pathway from the bulb is essential for olfaction and virtually all connections from the olfactory bulb must be destroyed to produce complete anosmia. The ability to discriminate between odors is more easily disrupted by rhinencephalic lesions, although large lesions of olfactory projection sites or rostral lesions of pathways must be made before severe deficits in discrimination occur. Because destruction of specific rhinencephalic areas (prepiriform cortex, olfactory tubercle, or amygdala) may be without effect on olfactory discrimination, it appears that large portions of the basal rhinencephalon are equipotential with regard to olfactory learning.

Proposed Course of the Project: To be terminated with the publication of the results.

Honors and Awards:

1) Lecture: "Functional neuroanatomy of the olfactory pathways," Department of Neurology, The Johns Hopkins Hospital, Baltimore, Maryland, March 12, 1974.

2) Lecture: "Studies in olfactory stimulus control," Department of Psychology, City University of New York (Queens College), Queens, New York, March 21, 1974.

3) Lecture: "Structure and function in olfaction," Department of Psychology, American University, Washington, D. C., April 23, 1974.

Publications:

Slotnick, B.M.: Fear behavior and passive avoidance deficits in mice with amygdala lesions. Physiology and Behavior, 11: 717-720, 1973.

Slotnick, B.M., Carpenter, M.L., and Fusco, R.: Initiation of maternal behavior prior to parturition in pregnant nulliparous rats. Hormones and Behavior, 4: 53-59, 1973.

Slotnick, B.M., and McMullen, M.F.: Passive avoidance deficits in mice with septal, amygdala or cingulate cortical lesions. Physiology and Behavior, 10: 385-389, 1973.

Slotnick, B.M., McMullen, M.F., and Fleischer, S.: Changes in emotionality following destruction of the septal area in albino mice. Brain, Behavior and Evolution, 8: 241-252, 1973.

Slotnick, B.M., and Katz, H.M.: Olfactory learning-set formation in rats. Science (in press).

Slotnick, B.M., and Nigrosh, B.J.: Maternal behavior in mice with cingulate cortical, amygdala, or septal lesions. Journal of Comparative and Physiological Psychology (in press).

Serial No. M-LBEB-CN-15
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Unit analysis of vagal inputs to the diencephalon. I. Intralaminar nuclei.

Previous Serial Number: Same

Principal Investigators: Robert A. Hallowitz and Paul D. MacLean

Other Investigators: Patrick J. Carr and Thalia D. Klosteridis

Cooperating Units: None

Man Years

Total:	1.6
Professional:	0.8
Other:	0.8

Project Description:

Objectives: Except for some earlier studies on evoked slow potentials almost nothing is known regarding the influence of visceral afferent systems on cerebral functions. The present study utilizes an improved technique for vagal stimulation in chronically prepared monkeys to test the effects of vagal volleys on units of midline and intralaminar nuclei of the thalamus.

Methods Employed: Extracellular unit potentials are recorded by an improved theta-glass micropipette (5-10 M) in awake, sitting squirrel monkeys prepared with a chronically fixed stereotaxic platform. Bipolar, concentric, stainless steel electrodes are chronically implanted in the jugular foramen next to the vagus and are led to a female plug on the platform. In addition to vagal volleys, units were tested for responsiveness to auditory, visual, and somatic stimulation.

Major Findings: More than 900 units were tested along 28 tracks in eleven squirrel monkeys. The majority of the tracks were ipsilateral to the stimulus. The most responsive areas were the paracentral nucleus and contiguous parts of lateral ventral

nucleus. The number of responsive units in these and other nuclei, together with the latencies for initially excited units, are listed in order as follows:

	No. and % Responding	Latencies for initially excited units	
		Range (msec)	Median
N. lateralis ventralis	23/71 (33%)	12-180	(20)
N. paracentralis	26/82 (32%)	12- 60	(20)
N. lateralis dorsalis	6/20 (30%)	13- 80	(20)
N. antero-medialis	5/17 (29%)	50	(50)
N. medialis dorsalis	97/413(23%)	16-200	(40)
N. centralis lateralis	5/32 (16%)	15- 20	(18)
N. centri mediani	9/61 (15%)	16-100	(22)
N. antero-ventralis	6/43 (14%)	17- 60	(40)
N. parafascicularis	2/23 (8%)	20- 80	(50)

No responsive units were found in the N. ventralis postero-medialis and a number of other non-intralaminar nuclei.

Ten percent of all units tested showed convergence and interaction of interoceptive and exteroceptive stimuli. Almost all of these cells were in medial and intralaminar nuclei.

Significance to Biomedical Research and Program of the Institute. For many years the paracentral nucleus has been recognized as one of the intralaminar nuclei belonging to the "diffuse, non-specific projection system" of the thalamus. The present study shows for the first time that it receives an input from the vagus nerve and suggests that it transmits visceral information to the cerebral cortex. Since there is evidence that it projects to the cingulate and other midline cortex, the results of the present study help to explain the findings of the microelectrode study described in Project M-LBEB-CN-2.

Proposed Course of the Project: To be continued.

Honors and Awards: None

Publications:

Hallowitz, R.A. and MacLean, P.D.: Effects of vagal volleys on unit activity of medial thalamic nuclei in squirrel monkeys (Saimiri sciureus). Fed. Proc., 33, 342, 1974.

Serial No. M-LBEB-CN-17
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Comparative study of aminergic systems of the brain.

Previous Serial Number: Same

Principal Investigators: David M. Jacobowitz and Paul D. MacLean

Other Investigators: None

Cooperating Units: Laboratory of Clinical Science
National Institute of Mental Health

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: This project addresses itself to the question of whether or not the three recognized aminergic systems of the brain originally described in the rat by Dahlström and Fuxe have a similar pattern of organization in the primate brain.

Methods Employed: The fluorescent technique of Falck and Hillarp is used for comparing the brains of the mouse, rat, pygmy marmoset, and squirrel monkey. Several hours before sacrifice tranylcypromine is administered for the purpose of enhancing fluorescence.

Major Findings: For mapping the location of cells and terminals the brain of the pygmy marmoset is particularly advantageous because its small size facilitates the handling of whole sections and their microscopic examination at closely spaced intervals.

Except for minor differences to be mentioned, the catecholamine (CA) containing cells (groups A1-A13 of Dahlström and Fuxe) were found in locations similar to those described in rat. CA cells in and surrounding the superior cerebellar peduncle are more conspicuous in the marmoset than the rat. They probably belong to the same system of cells identified as A6 and A7. CA cells seen ventrolateral to the medial longitudinal fasciculus at the level of the trochlear nucleus may represent a forward extension of A6 cells of the locus coeruleus. Small CA cells scattered along the periaqueductal gray possibly belong to either group A6 or A11. These cells may be the origin of processes terminating in the periventricular nucleus of the caudal thalamus and at sites close to the lateral habenular nucleus. Unlike the rat and squirrel monkey, no A13 cells were seen between the mammillothalamic tracts.

The fluorescent technique allows a clear separation of the locus coeruleus and the lateral dorsal tegmental nucleus, two structures which in the past have often been considered as parts of the same nucleus.

Only one detail need be added to the description in last year's report of the distribution of CA terminals: From the level of the medulla to the zona incerta fine vericosities were seen in locations corresponding to the course of the central tegmental fasciculus. This finding suggests that the various groups of norepinephrine containing cells may represent a unified system associated with this phylogenetically ancient fasciculus.

As reported last year, serotonin containing cells were found in raphe nuclei corresponding to those in the rat.

Significance to Biomedical Research and Program of the Institute. The recently discovered aminergic systems of the brain are currently of great interest because of evidence of their implication in mental illness. The present studies have revealed that the pattern of organization of these ancient systems of the brain have been preserved with remarkable consistency in the evolution of primates.

Proposed Course of the Project: To be terminated with the publication of the results.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-24
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neural substrate of nesting behavior of mouse I. Corpus striatum.

Previous Serial Number: Same

Principal Investigators: Burton M. Slotnick, Robert E. Gelhard, and Paul D. MacLean

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.55
Professional:	0.15
Other:	0.4

Project Description:

Objectives: The present project is part of an investigation testing the hypothesis that the corpus striatum plays a basic role in species-typical behavior. As described in last year's report, it was found that in mice primed with progesterone (to stimulate nest building) small lesions in the head of the caudate nucleus resulted in a 77% decrease in the retrieving of thread given as nesting material. The present study extends these findings for mice with larger caudate lesions and more extensive tests for nest building.

Methods Employed: Ten adult NIH-GP strain female mice were individually housed and given free access to 3-ply cottong string for nesting material. Pilot studies showed that non-pregnant mice reliably retrieve and build nests with the string without priming with exogenous progesterone. After a control period, animals were subjected to sham lesioning procedures, and 30 days later an electrocoagulation was made in the head of the caudate nucleus on one side in 9 mice. Forty-five days later lesions

were placed in the contralateral nucleus. One mouse with large neocortical lesions served as a control. Prior to sham lesioning procedures and again before termination of the experiment each mouse was also given 3-4 tests of its ability to build a nest with three 1-inch long pieces of multiple stranded hemp twine. Nests were rated on a 0-4 scale.

Major Findings: Four mice subjected to large lesions of the caudate nucleus on one side died shortly after operation.

The average nest weight, average percent change in nest weight, and ratings of nest construction are given in the following table:

	Before Surgery	After Sham Lesions	After First Caudate Lesion	After Second Caudate Lesion
String Used (gm.)	10.	9.4	3.1	2.5
$\bar{X}$ % Change		-2%	-75%	-78%
Nest Ratings with Twine	3.1 (2.5-3.6)			3.1 (2.9-3.6)

Unilateral caudate lesions resulted in a marked decrease in retrieving string in each mouse. After the second lesion there was a further but much smaller decrease in retrieval. However, nest building tests revealed no deficits in ability to build nests when the mice were given a fixed quantity of hemp twine. Both before and after surgery each mouse built a small or medium sized nest by first separating the twine and then "weaving" the strands into a cup-shaped form. The neocortical lesioned control animal showed no change in retrieving of string or nest building.

Histological examination of the brain revealed that the striatal lesions were placed bilaterally in the head of the caudate nucleus. Comparisons among animals indicated that the magnitude of the deficit in retrieving string was related to the size of lesion: The 2 mice with the smallest lesions showed the least change in string retrieving (-57 and -67%), while the 2 mice with the largest lesions showed the greatest deficits (-89 and -98%).

Significance to Biomedical Research and Program of the Institute. The present study confirms our previous finding that lesions in the head of the caudate nucleus in mice results in a profound deficit in retrieving of nesting material but not in the ability to construct a nest. This lesion-induced deficit has now been observed both in mice hormonally primed to induce nest building and in non-hormonally primed mice tested with a material (cotton string) of high incentive value for nesting. Differences in lesion-induced effects between retrieving nesting material and ability to construct a nest indicate that these 2 behavioral processes may be independently affected and that striatal lesions of moderate size may specifically affect the "appetitive" (retrieving of nesting material) but not the "consumatory" (nest construction) aspect of this species-typical behavior.

Proposed Course of the Project: To be continued.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-25
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Projections of the olfactory bulb.

Previous Serial Number: M-LBEB-CN-8

Principal Investigators: Burton M. Slotnick and Paul D. MacLean

Other Investigators: Thalia D. Klosteridis

Cooperating Units: None

Man Years

Total:	0.55
Professional:	0.3
Other:	0.25

Project Description:

Objectives: Anatomical studies indicate that the lateral olfactory tract is the principal, and perhaps only, pathway from the olfactory bulb to the forebrain. Behavioral studies, however, described in last year's report, demonstrated significant sparing of olfactory function after ablations of areas innervated by the lateral olfactory tract and even after section of the tract at levels close to its origin. In the present study, improved anatomical techniques were used in an attempt to demonstrate other connections of the olfactory bulb with the forebrain.

Methods Employed: In adult mice the rostral portion of the right olfactory bulb was removed by aspiration. In other animals, lesions were placed stereotaxically in the right anterior olfactory nucleus. The animals were sacrificed 3-8 days after surgery, and 20-micron thick frozen sections through the forebrain were stained with either the Fink-Heimer or Voneida modification of the Nauta method.

Major Findings: The surgical procedure destroyed the rostral one-third to one-half of the bulb and produced varying amounts

of damage in pars rostralis of the anterior olfactory nucleus. The distribution of coarse and pre-terminal degeneration associated with the lateral olfactory tract was similar to that described in previous studies on rats, rabbits and guinea pigs. When degeneration was allowed to proceed for 7-8 days two additional groups of fibers were seen:

1. Coarse fibers arising from the ipsilateral anterior limb of the anterior commissure were traced toward the underlying medial portion of the pre-piriform cortex and lateral part of the olfactory tubercle.

2. Some degenerating fibers projected onto the ventromedial wall of the hemisphere ipsilateral to the lesion and rostral to the genu of the corpus callosum. Terminal degeneration associated with these fibers was most prominent in the outer part of the plexiform layer of the hippocampal rudiment.

Both of these patterns of degeneration were also found in animals with lesions destroying the anterior limb of the anterior commissure and portions of the pars dorsalis and pars medialis of the anterior olfactory nucleus. In these cases no degeneration was observed in the lateral olfactory tract.

Significance to Biomedical Research and Program of the Institute. The present findings in the mouse have confirmed the existence of heretofore questionable projections from the ipsilateral limb of the anterior commissure, as well as olfactory terminations in the hippocampal rudiment. Although it is not possible to conclude whether these projections originate from the olfactory bulb or from the anterior olfactory nucleus, both pathways could account for sparing of olfactory function after destruction of the lateral olfactory tract.

Proposed Course of the Project: To be terminated with the publication of the results.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-26
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Evaluation of peripheral anosmia produced by intranasal treatment with zinc sulfate.

Previous Serial Number: None

Principal Investigators: Burton M. Slotnick and Lois A. Gutman

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.1
Other:	0.6

Project Description:

Objectives: Recent studies have indicated that deficits in rodent social behavior produced by removal of the olfactory bulbs are not obtained after peripherally induced 'anosmia' (intranasal treatment with 1 cc of zinc sulfate). These studies have cast doubt on the presumed dependence of these behaviors on olfaction and suggest that the olfactory bulb provides a significant non-olfactory influence on brain activity and behavior. However, there has not been an adequate evaluation of zinc sulfate treatment in producing anosmia. The present study was designed to test the extent of olfactory deficits produced by zinc sulfate treatment.

Methods Employed: Eight rats were trained in a wind-tunnel olfactometer with the use of a discrete trials "go, no-go" discrimination procedure requiring the detection of the presence of amyl acetate (0.1% dilution) and a discrimination between pairs of amyl acetate dilutions with difference ratios of 0.5, 0.25 and 0.16, respectively. Animals were treated by intranasal syringing with isotonic saline (4 rats), 1 cc or 6 cc of zinc sulfate (2 rats each). Post-treatment tests on each task were

given every 48 hours until performance returned to its pre-treatment level. As an additional control, animals were trained and tested on a simple visual discrimination problem.

Major Findings: Intranasal treatment with saline had no effect on olfactory discrimination. After treatment with zinc sulfate animals had perfect retention of the visual discrimination but were unable to detect the odor of 0.1% amyl acetate for 1-7 days and had progressively greater deficits for the more difficult odor intensity tasks. Both the 1 cc and 6 cc treatments produced equivalent deficits. Table 1 summarizes the recovery of function for each of the experimental animals. In preceding studies olfactory bulbectomy was found to produce complete and permanent loss in odor detection.

Table 1. Days required for performance to return to pretreatment level after intranasal syringing with zinc sulfate.

	Task (Odor intensity difference ratio)			
Rat	Detection-only	0.5	0.25	0.16
134	1	3	3	7
136	5	5	7	7
125	3	5	5	7
123	7	7	9	9
Mean	4	5	6	7.5

Significance to Biomedical Research and Program of the Institute. Intranasal treatment with zinc sulfate significantly depresses olfactory function but the duration of anosmia (inability to detect odors) is brief and variable among animals. These effects on olfaction are obviously not equivalent to those produced by removal of the olfactory bulbs. The results suggest that the differences in behavior obtained in prior studies comparing olfactory bulbectomy with peripherally induced 'anosmia' may be confounded by residual olfactory function after the latter treatment. Convincing demonstration for a "non-olfactory" function of the olfactory bulbs will require a more effective procedure for producing anosmia at the receptor level.

Proposed Course of the Project: The project will be terminated with the publication of the results.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-27
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Odor detection and discrimination after intraperitoneal injections of DL-p-chlorophenylalanine.

Previous Serial Number: None

Principal Investigators: Burton M. Slotnick, David S. Bachman, and Howard M. Katz

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.15
Professional:	0.1
Other:	0.05

Project Description:

Objectives: The present experiment examines the effects of DL-p-chlorophenylalanine (PCPA) induced depletion of brain serotonin on olfactory function. Previous findings that both treatment with PCPA and removal of the olfactory bulbs produce striking increases in rat aggressive behavior suggests that PCPA may alter olfactory function.

Methods Employed: Rats were trained in a wind tunnel olfactometer using a discrete trials, go, no-go procedure. After determining the odor detection threshold for successive dilutions of amyl acetate (concentration range: 10^{-3} to 10^{-7} in half log unit steps) 2 rats were given intraperitoneal injections of 100 mg/kg of PCPA on each of two days and a 200 mg/kg injection on the third day. Eighteen hours after the last PCPA injection the odor detection threshold was determined again. Two other rats were treated in a similar manner except for being trained to discriminate the odors of ethyl acetate from isopropyl acetate. The minimal stimulus duration for discrimination was determined in a pre- and post-treatment session by decreasing stimulus

duration from 1 to 0.2 sec in 0.2 sec steps.

Major Findings: Treatment with PCPA had little or no effect on odor detection or discrimination performance. Performance of the two animals trained on odor detection was similar, and detection threshold both before and after treatment was between the 5×10^{-6} and 10^{-7}. Prior to PCPA treatment the two animals trained on the 2-odor problem showed significant discrimination on all but the shortest (0.2 sec) stimulus durations. After treatment, their performance was similar but accuracy was slightly lower on each of the stimulus durations tested.

Significance to Biomedical Research and Program of the Institute. Since both the detection and discrimination tasks encompassed a range of difficulty from suprathreshold to below threshold, the failure to find an olfactory deficit is probably not due to insensitivity of the tests. The results indicate that behavioral alterations produced by PCPA-induced depletion of brain serotonin are not secondary to disruption of olfactory function.

Proposed Course of the Project: The project is terminated.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-28
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A stereotaxic brain atlas of the albino mouse.

Previous Serial Number: None

Principal Investigators: Burton M. Slotnick

Other Investigators: Christiana Leonard, Rockefeller University

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: The aim of the present project was to prepare a stereotaxic atlas of the albino mouse brain for neurological investigations.

Methods Employed: Adult male albino mice of the CF-1 strain were anesthetized and killed by cardiac perfusion with saline and 10% formalin. The perfused heads were mounted in a specially designed head holder fixed in a stereotaxic machine. The horizontal plane was defined as a line passing through the bregma and lambda. The vertical zero coordinate was defined by the height of the skull at the bregma, the frontal zero by the anterior-posterior position of bregma, and lateral zero by the sagittal suture. Tracks for marking stereotaxic coordinates of the brain were made by inserting electrodes and making small electrolytic lesions at appropriate coordinates. Brains were sectioned in the frontal, horizontal, or sagittal plane at 30 or 40 μ according to the frozen section method of Marshall.

Major Findings: One brain cut in the frontal plane and one cut in the sagittal plane were selected for illustrating the atlas.

The stained sections of these brains had less than 5% shrinkage and were representative of a series of 27 brains used to obtain an estimate of stereotaxic variability.

The usefulness of a stereotaxic atlas depends on the accuracy with which brain targets can be localized. An analysis revealed that variation in the vertical and horizontal planes of specific structures is relatively small (mean standard deviation of all measures: 0.185 mm). Significantly greater variability (0.256 mm) was found in the frontal plane. It was found that a measure of skull size (distance between bregma and lambda in the frontal plane) was significantly correlated with the frontal position of neural structures. A correction factor based on this relationship provided a significant reduction in frontal variability. With the present atlas, it is estimated that subcortical lesions may be made in the mouse brain with accuracy equivalent to that achieved with rats.

Significance to Biomedical Research and Program of the Institute. Mice are excellent subjects for investigations of learning and species-typical behaviors. Their small size and social habits make them ideal subjects for studies of group dynamics, crowding, and population genetics. However, the absence of a stereotaxic atlas has limited the use of these animals in neurophysiology, neuropsychology, and experimental histochemical studies. The present atlas provides a guide for placing electrodes for stimulation, recording, and for making lesions.

Proposed Course of the Project: The atlas is now being prepared for publication in book form by the Government Printing Office and will consist of photomicrographs of Nissl stained sections both through the frontal plane (at 0.1 mm intervals) and sagittal plane (at 0.3 mm intervals).

Honors and Awards: None

Publications:

Slotnick, B.M. and Leonard, C.: A stereotaxic atlas of the albino mouse forebrain. Government Printing Office (in press).

Serial No. M-LBEB-CN-29
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Role of corpus striatum in species-typical behavior of canids.

Previous Serial Number: None

Principal Investigators: Paul D. MacLean

Other Investigators: Stephen C. Barker

Cooperating Units: None

Man Years

Total:	0.15
Professional:	0.05
Other:	0.1

Project Description:

Objectives: The corpus striatum (caudate + putamen) in mammals is a large ganglionic mass which represents a major counterpart of the reptilian forebrain. Its functions are unknown. It sends massive projections to the globus pallidus, which has been found in this laboratory to be implicated in the species-typical behavior of squirrel monkeys. Canids may prove valuable for the investigation of striatal functions because they have an unusually large caudate nucleus and because they demonstrate not only species-typical, but also breed-typical, behaviors. The purpose of the present experiments is to ablate the caudate in immature animals and then to observe the capacity of members of a particular breed to develop their usual behavioral repertoire.

Methods Employed: A first requisite for the proposed study is to learn whether or not it is feasible to ablate the entire caudate nucleus. Acute experiments dealing with this matter constitute the major findings of the present report.

Major Findings: Acute experiments for working out the operative approach have been conducted on six animals ranging in age from four weeks to four months. It appears that the risk of mortality from administering anesthesia to immature animals can be averted by the use of a tracheal cannula and a volatile anesthetic such as Halothane or nitrous oxide. The caudate nucleus extends too far laterally to permit a midline approach through the corpus callosum and to aspirate it with suction. Since electro-coagulation by a stereotaxic approach is undesirable because of complications due to swelling and other factors, it is planned next to attempt aspiration by the stereotaxic technique.

Significance to Biomedical Research and Program of the Institute. The corpus striatum constitutes a large central mass in the forebrain of all mammals. The traditional clinical view that it serves as part of the motor system is not supported by findings that large bilateral lesions may result in no motor deficits. Previous work in this laboratory supports the hypothesis that it plays a fundamental role in species-typical behavior. Its high content of cholinesterase, dopamine, and serotonin makes the striatum of unusual interest with respect to the use of psychotropic agents. It has also been reported to have high opiate binding. There is evidence that it may be implicated in the amphetamine psychosis.

Proposed Course of the Project: To be continued.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CN-30
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Reptilian forebrain mechanisms of stereotyped and species-typical behavior. I. Ethological methods.

Previous Serial Number: None

Principal Investigator: Neil B. Greenberg

Other Investigators: Robert Gelhard, Patrick J. Carr, and Jeanna Goffredi

Cooperating Units: Section on Behavioral Systems, Laboratory of Brain Evolution and Behavior; Technical Development

Man Years

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

Objectives: In recent years, histochemical studies have provided clarification of corresponding forebrain structures in reptiles, birds, and mammals. Since reptiles show stereotyped and species-typical behavioral patterns, we plan to use them for obtaining clues regarding the functions of the striatal complex, hippocampal formation, and amygdala, as well as the recently discovered aminergic systems of the brain. Before undertaking such investigations it is necessary: (1) to select species with well developed behavioral patterns that are suitable for experimental study; (2) to obtain a complete profile of their normal behavior (ethogram); and (3) to devise methods for quantifying the observed activities.

Methods Employed: Reptiles selected for evaluation are of the suborder Sauria (lizards), and include members of both the Autarchoglossid group (generally specialized for olfaction) and the Ascalabota group (specialized for vision).

(1) For preliminary observations animals are kept in enclosures designed for manipulation of vital parameters such as type of prey and its availability, heat, light, and other physical aspects of the habitat. (2) Data thus obtained are then combined with field observations and other information for constructing appropriate experimental habitats. (3) The "observer effect" (presence of an observer interfering with the lizard's expression of natural behavior) is circumvented by the use of: (i) closed circuit television monitoring supplemented by time lapse videotape analysis; (ii) electromechanical monitoring of animals' movements; and (iii) radiotelemetry for the study of thermoregulatory behavior.

Major Findings: To date, representatives of twelve genera have been studied. Table 1 gives a rating of their suitability for the studies under consideration. Commercial availability and body size were additional factors in the choice for the present project of the four species listed in the last column.

Preliminary ethograms reveal widely varying preferences among the different species in the selection of microhabitats for sleeping, basking, and perching, as well as different strategies of behavioral thermoregulation, predation, and social organization. The following behavioral patterns appear not to have been previously reported in the literature: mutual grooming (Anolis carolinensis); social facilitation of feeding (Agama agama, Anolis carolinensis, Sceloporus cyanogenys); and social learning (seen in Sceloporus cyanogenys, an insectivore that began to eat lettuce after observing a vegetarian cohabitant, Dipsosaurus).

Significance to Biomedical Research and Program of the Institute. A prerequisite for investigations of brain mechanisms of reptilian behavior is the selection of species with behavioral patterns that can be reliably elicited under controlled conditions. The present survey indicates that the common green lizard (Anolis carolinensis), the rainbow lizard (Agama agama), the desert lizard (Dipsosaurus dorsalis), and the common iguana (Iguana iguana) will be useful for special kinds of brain research.

Proposed Course of the Project: To be continued.

Honors and Awards: None

Publications: None

Table 1. Experimentally suitable for listed conditions and behaviors.

	Retention of Feral Behavior	Observer Effect	Spontaneous Activity	Thermoregulatory Behavior	Predatory Behavior	Social Interaction	Territorial Behavior	Display Behavior	Aggressive Behavior	Defensive Behavior	Courtship and Mating	Brain Study
Agama agama (110mm)	4	3	4	3	4	4	4	4	3	3	3	A
Ameiva ameiva (150mm)	3	1	2	3	3	-	-	2	-	3	-	
Anolis carolinensis (50mm)	4	4	4	2	4	4	4	4	3	4	4	H
Anolis distichus (50mm)	4	4	4	2	4	3	-	4	-	-	-	
Anolis equestris (150mm)	1	1	2	2	3	2	3	2	2	3	-	
Anolis garmani (130mm)	3	3	3	2	4	4	4	4	3	3	-	
Dipsosaurus dorsalis (115mm)	3	4	3	4	3	1	1	2	1	1	-	A
Eumeces inexpectatus (75mm)	3	1	3	3	4	-	-	-	-	3	-	
Gekko gecko (160mm)	3	2	1	1	3	-	-	-	3	4	-	
Iguana iguana (400mm)	2	3	2	2	2	2	2	2	2	4	-	A,S
Sceloporus cyanogenys (125mm)	4	3	3	3	4	4	4	4	3	3	3	
Sceloporus orcutti (115mm)	3	2	3	3	3	3	4	3	2	3	-	

1=poor
2=fair
3=good

4=excellent
- =not observed

S=Brain Stimulation
A=Brain Ablation
H=Histochemistry

Serial No. M-LBEB-CN-31
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Neurophysiology and Behavior
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Thermoregulatory behavior of lizards.

Previous Serial Number: None

Principal Investigator: Neil B. Greenberg

Other Investigators: Patrick J. Carr and Jeanna Goffredi

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description:

Objectives: Lizards regulate their body temperature by moving into or away from a source of heat. Since their activity in social and other situations is influenced by body temperature, it is necessary for studies on brain mechanisms of reptilian behavior to have methods for detecting the retention or loss of thermoregulatory behavior. The present study tests the relative influences of light and heat in affecting behavior.

Methods Employed: Lizards of four species have been observed in feral maintenance enclosures in which their behavior is uninfluenced by the presence of an observer and in which heat and light can be independently varied. Body temperatures are measured either by a quick-reading electronic thermometer or implanted temperature sensitive radiotelemeters.

Major Findings: The anolis lizard (Anolis carolinensis) was studied by using a proximity sensor to monitor movements into a small spot of light in a dim habitat and providing thermal reinforcement only when the animal was in the light. The association was slow to develop and extinguished rapidly without reinforcement. Desert iguanas (Dipsosaurus dorsalis), however,

acquired the association rapidly and were slow to extinguish.

Given a choice of a bright cooler site or a warmer dimmer site (5° gradient) the common iguana (Iguana iguana) selects the warmer place for basking. One animal repeatedly interrupted basking and compromised the effectiveness of its thermoregulation in order to displace a potentially competitive conspecific from basking at the alternate site, leaving the subordinate animal with no source of heat.

Rainbow lizards (Agama agama) were studied in an enclosure in which morning light preceded heat by two hours. They did not leave their sleeping site until thermal reinforcement was available. Desert iguanas, however, regularly emerged in response to the morning light before heat became available.

Significance to Biomedical Research and Program of the Institute: Thermoregulatory behavior of reptiles affects all other behavior. The present study has shown that some species of lizards are differentially affected by light and heat. These findings provide necessary information for studies of brain mechanisms of reptilian behavior.

Proposed Course of the Project: To be continued.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-7
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Attachment behavior in beagle dogs as a function of social reinforcement.

Previous Serial Number: Same

Principal Investigators: Walter C. Stanley and Stephen C. Barker

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.41
Professional:	.20
Other:	1.21

Project Description:

Objectives: To contribute to our understanding of social attachment by identifying and studying sources of reinforcement inherent in conspecific and interspecific social situations. The relative reinforcing values of visual vs free access to the following stimuli were determined: a conspecific stimulus (dog), an interspecific stimulus (passive person), and for comparison, non-social stimulus objects.

Methods Employed: Thirty-six beagle dogs were run in a T-maze. They received six pretraining trials, two trials per day, beginning when they were 56-61 days old, then 25 training trials, one trial per day, five days per week. In each of three experiments, four dogs had (contingent on a correct choice in the T-maze) free access for one minute to the stimulus object(s) in the goal area (Plexiglas barrier absent); four dogs had primarily visual contact for one minute with the stimulus object(s) in the goal area (Plexiglas barrier present); and four dogs were confined for one minute in the goal area (Plexiglas barrier absent for two dogs and present for two dogs). The interspecific object (Exp. 1) was

a person (the experimenter) wearing a white lab coat and sitting passively in the goal area. The conspecific object (Exp. 2) was a littermate tethered in the goal area. The nonsocial objects (Exp. 3) were a tennis ball and artificial bone together in the goal area. On each trial, the experimenter recorded the dog's choice (right or left), and three response times (starting, running, and goal area response times).

Major Findings: With free access to the goal stimulus, both a conspecific and an interspecific were highly effective reinforcers. Free access to the inanimate objects was not reinforcing. With visual contact, the conspecific and interspecific objects were reinforcing, although choice and speed of running were somewhat poorer than those obtained with free access to goal stimuli. In general, the present data (a) confirm earlier findings on the role of passive person reinforcement in canine attachment behavior, (b) justify the extension of an instrumental reinforcement (learning) analysis to dog-dog interactions, (c) demonstrate that the obtained person and dog reinforcement effects were not based on properties generally characteristic of inanimate objects, and (d) indicate that a visual social stimulus has reinforcing value.

Significance to Biomedical Research and Program of the Institute. Research on social reinforcement inherent in interspecific and conspecific social interactions should lead to the discovery of new principles applicable to the study of human social behavior. For example, the fact that a passive person and a canine cagemate appear to be equivalent reinforcers in young beagles suggests that availability for many interactions, rather than occurrence of one particular interaction, is the main requirement for socialization of the young. However, after socialization, as an active process, has taken place the particular interactive patterns characteristic of the reinforcer are those the young animal "seeks."

Proposed Course of the Project: The project is terminated. A preliminary report of this year's findings has been accepted for oral presentation at the American Psychological Association meetings in New Orleans, and reports for journal publication of this and previous year's findings on this project are in preparation.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-8
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Behavioral mathematics and the logic of measurement.

Previous Serial Number: Same

Principal Investigator: Walter C. Stanley

Other Investigators: None

Cooperating Units: None

Man Years

Total:	.16
Professional:	.10
Other:	.06

Project Description:

Objectives: To devise a logic and mathematics uniquely appropriate to individual behavior as it occurs sequentially in real space and real time, in natural as well as in artificially contrived settings.

Methods Employed: These were mathematical analyses (in general form) of theoretically conflictual behavioral situations wherein strong approach and withdrawal oppose each other as, for example, in the socialization of people and mammals and in the imprinting of birds.

Major Findings: Changes in behavior-enhancing and behavior-suppressing variables may be directly and immediately reflected in overt behavior. When this happens, negative, zero, and positive numbers (the "real" numbers) suffice for measurement. However, when conflictual variables produce no immediately correlated behavior, it appears that complex numbers ($\underline{a} + \underline{bi}$) composed, respectively, of a real number component $\underline{a}$ and an imaginary number component ($\underline{bi}$), are required to represent

mathematically and quantitatively the motivational status of the conflict and its potential outcome in behavior.

Significance to Biomedical Research and the Program of the Institute. Confirmation of the validity of using complex numbers to represent the variables in conflict situations would extend the methods of quantitative behavioral science to analyses of human behavioral problems now largely conceptualized only qualitatively or mentalistically.

Proposed Course of the Project: To be continued until the mathematical analyses are published in the context of relevant evaluative research findings.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-CB-16
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ontogeny of early learning

Previous Serial Numbers: M-LBEB-CB-1, 2, 4, 9, 13, and 14.

Principal Investigator: Walter C. Stanley

Other Investigators: E. Faye Bacon, Stephen C. Barker, Gerson Grosfeld, Stephen N. Lee, Rodney M. Sewell

Cooperating Units: None

Man Years

Total:	1.75
Professional:	.25
Other:	1.50

Project Description:

Objectives: To evaluate the effectiveness of appetitive and aversive reinforcement schedules in neonatal and infant mammals as a function of the age of the subjects and the manner of their rearing (dam-vs. other-than-dam-rearing). The project intercalates with Project Number M-LBEB-CB-17 on the "Ontogeny of Adaptive and Maladaptive Adjunctive Behavior," and combines in a single and more economical project selected parts of six projects of the Section.

Methods Employed: These include (a) discrete trials instrumental conditioning of crawling based on appetitive (milk) and aversive (cold air stream) reinforcement; (b) discriminated conditioning of crawling on tactual discriminative stimuli; (c) operant conditioning of sucking with milk reinforcement; and (d) operant conditioning of key lifting with milk reinforcement.

During the past year we sought additional evidence concerning the capacity of neonatal and infant dogs to inhibit natural consummatory behavior (sucking) and operantly conditioned behavior (key

lifting). Four 5-7 and four 12-14 day-old beagle puppies were trained to suck on an artificial nipple. Milk reinforcement was scheduled to be differential with respect to rate of response. In half the subjects sucks spaced 0.5, 1.0, and 1.5 or more seconds apart were followed by decreasing amounts of milk; in the other half of the subjects, the same spacings of sucks were followed by increasing amounts of milk. Sucks spaced more closely than 0.5 seconds apart, were not followed by milk. Such differential reinforcement of low rate of response (DRL) schedules, it should be noted, penalizes the subject for fast responding because fast responding is never immediately followed by milk. If single sucks are spaced out in time, however, the same DRL schedules reduce to continuous reinforcement (CRF) because milk comes after every response.

In the key lifting situation, four 9-24 day-old beagle puppies were run, two with DRL schedules of milk reinforcement and two with fixed interval (FI) schedules. In the FI schedules, reinforcement is contingent on the first response that occurs a fixed time after reinforcement regardless of intervening behavior. The FI schedules, therefore, do not penalize subjects for high rate of responding as the DRL schedules do.

In another experiment of this series, two 2-7 and two 5-10 day-old dam-fed beagle puppies were compared with their four machine-fed litter mates in five days of instrumental avoidance-of-cold-air-stream learning.

Major Findings:

1. In operant study of sucking, the older puppy run on an increasing DRL schedule had the highest sucking rate while the other three subjects had about the same low sucking rate.

2. In operant key lifting, the DRL schedule slowed rate of response more than the FI schedules did, but persistance of differential effects were transient and small in magnitude when all subjects received milk after every key lift.

3. In three matched pairs of puppies, avoidances were markedly more frequent in machine- than in dam-fed animals; in one matched pair, avoidances were slightly more frequent in the dam- than in the machine-fed animal. Machine-fed animals did not gain body weight as rapidly as the dam-fed animals did; hence they were probably generally more hungry and active.

Significance: The slowness and transience of inhibitory conditioning is generally regarded as an important characteristic of learning in neonates and infants. The present findings on differential reinforcement of low rate of sucking and key lifting indicate that the lack of early inhibition is relative rather than absolute and specific to particular learning situations. This may explain the fact that an occasional neonatal dog is an excellent inhibitor of both consummatory and operantly conditioned behavior. The findings on avoidance performances of machine- vs. dam-fed infant puppies suggest that immediate behavioral effects of early experiences cannot be assigned to some global experiential effect on learning in general. Rather particular experiences seem to have particular effects in particular learning situations.

Proposed Course: To be continued insofar as subjects assigned to Project No. M-LBEB-CB-17 can also be used in the present project.

Honors and Awards: For items pertaining to Dr. Stanley, see listing under Project No. M-LBEB-CB-17.

Publications:

Stanley, W.C., Barrett, J.E., and Bacon, W.E.: Conditioning and extinction of avoidance and escape behavior in neonatal dogs. J. Comp. Physiol. Psychol., in press.

Serial No. M-LBEB-CB-17
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ontogeny of Adaptive and Maladaptive Adjunctive Behavior.

Previous Serial Numbers: M-LBEB-CB-3, 9, 10, 15

Principal Investigator: Walter C. Stanley

Other investigators: E. F. Bacon, Gerson Grosfeld, Stephen N. Lee

Cooperating Units: None

Man Years

Total:	1.83
Professional:	.25
Other:	1.58

Project Description:

Objectives: To study adjunctive behavior in infant and adult animals. Adjunctive behavior is behavior unnecessary for the occurrence of reinforcement in a learning situation. For example, a dog obtaining food by lifting a key may develop systematic ways of licking an empty food dish and walls of the apparatus. If the adjunctive licking delays key lifting when key lifting is inappropriate (i.e., will not be reinforced), then licking is adaptive; if licking delays key lifting when key lifting is appropriate (i.e, will be reinforced), then licking is maladaptive. This project intercalates with Project Number M-LBEB-CB-16 on "Ontogeny of Early Learning," and combines in a single more economical project selected parts of four previous projects.

Methods Employed: Eight adult beagle dogs were run with dry food reinforcement in operant chambers controlled by the Section's computer (LINC). Frequency of key lifting was continuously recorded. Interresponse times of key lifting and samples

of adjunctive behavior (vocalization, water drinking, and panel licking and pawing) were recorded on an intermittent basis. As infants, four of these beagle dogs had been permanently removed from the dam and started in operant conditioning with milk reinforcement, two at 6 and two at 16 days of age, and four dogs had been removed from the dam, hand fed, but not operantly conditioned, two at 5 and two at 15 days of age.

Three of the four puppies operantly conditioned to key lift during the present year (Project No. M-LBEB-CB-16, FY 1974) have been returned to the dam and are being reared as an intact litter with two littermates never removed from the dam. Each member of the litter will be run as adults in the operant conditioning chambers.

Major Findings: The key lift data from this project are now being tabulated and systematically graphed. Analysis of such behavior is required for valid interpretation of the behaviors adjunctive to key lifting. Inspection of preliminary analyses permit some statements concerning general trends in the data:

1. There was no real overall group difference in adult key lifting performance between dogs trained to key lift to feed in infancy and control dogs fed by hand but not trained in infancy. As adults the infant-trained dogs key lifted to quota for the first time during Session No. 20.75 on the average, while the infant-fed dogs did so during Session No. 19.75; the two groups attained a predetermined performance criterion, respectively, on Session No. 41.00 and 42.75 and thus required, respectively 20.50 and 23.00 sessions to meet the criterion once they started key lifting.

2. In contrast, there was a marked difference in starting to perform in adult key lifting between dogs removed earlier (5 or 6 days old) vs. later (15 or 16 days old) in infancy. Once the earlier group started to perform, however, they achieved criterion as rapidly as the later group, indicating that the behavioral deficit pertained only to starting to perform appropriately and not to learning in general. Dogs removed earlier in infancy key lifted to quota for the first time on Session No. 27.25, dogs removed later in infancy, on Session 13.25; the two groups attained criterion, respectively, on Session No. 47.00 and No. 36.75 and required 19.75 and 23.50 sessions to attain criterion once they started to key-lift.

3. The findings on adult performance in dogs with earlier vs. later removal from dam during infancy are in general agreement with the findings in the first litter of four animals run (Project Number M-LBEB-CB-10, FY 1973). In this first litter, adult dogs removed earlier in infancy key lifted to quota for the first time on Session 27.50, dogs removed later in infancy, on Session 10.00; the two groups attained criterion, respectively, on Session No. 43.50 and No. 35.50 and required 16.00 and 26.50 sessions to attain criterion once they started to key lift.

4. As adults the earlier removed dogs were also generally more timid, especially in novel situations, and required more stimulation by the experimenter and special handling ("behavioral therapy") to start to eat and to key lift in the apparatus. In each of the three litters studied, one of the two earlier removed dogs was markedly deviant in such timidity. Two were moderately deviant, and one (in the infant-fed litter) was a good steady performer until he had a fight with the other male in his litter. The immediate effect of the fight was "choppy performance with many breaks and 'howling'". Thereafter he settled down to steady but somewhat slow performance. Of the six later removed dogs, four were excellent performers and two were slightly hesitant.

5. Any finding on the effects of early experience on adult behavior must be interpreted with care. Factors correlated with differential early experience (anatomical and physiological as well as behavioral) might account for the later behavior. In the present project, markedly deviant behavior was also correlated with retardation in body weight gain produced by early removal from the dam in infancy. The nutritional factor might account for the deviant adult behavior either directly through anatomy or indirectly through lighter animals being dominated by their heavier littermates. Such domination might have rendered the earlier removed animals generally more timid than later removed animals.

Significance: The manner in which early experience and nourishment affects later behavior is a fundamental issue with respect to human intelligence as well as human behavioral development. The finding that observed adult behavioral deficits in dogs cannot be ascribed directly to early learning experiences, qua learning experiences, but are apparently due to correlated factors (such as slowed weight gain and domination by littermates)

is in general agreement with recent findings of others. Unless there are irreversible anatomical changes, apparently detrimental early experience can be corrected by later more appropriate experience. Conversely, early experience not detrimental in itself may lead to later conditions which are detrimental.

Proposed Course: To be continued.

Honors and Awards: Items pertaining to Dr. Stanley are as follows:

1) Invited to make site visit for evaluating the scientific merits of the behavioral parts of a large-scale study on canine physiology and behavioral functioning, December, 1973.

2) Invited panel member: "Animal Behavior and Intelligence." Station FM WAMU, American University, Washington, D.C., March 19, 1974.

3) Invited seminar speaker: "Learning and Infancy." Department of Psychology, George Washington University, March 21, 1974.

4) Invited seminar speaker: "Animal Behavior and Intelligence." Conference on American Civilization, Montgomery County Schools, April 10, 1974.

5) Appointed member: Committee on Animal Experimentation, American Psychological Association.

6) Invited interviewee: On "Ethology" for educational film on Section's early learning research, WETA-TV. It is estimated that the film will be seen by 7,000,000 junior high school students in the next seven years.

Publications:

Stanley, W.C.: Motor abnormalities in infant dogs induced by intermittent oral stimulation. In Bosma, J.F. (Ed.), Fourth symposium on oral sensation and perception: development in the fetus and infant. U. S. Government Printing Office: Washington, D.C., in press.

Serial No. M-LBEB-CB-18
1. Laboratory of Brain Evolution and Behavior
2. Section on Comparative Biopsychology
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974.

Project Title: Behavior Genetics: Ontogeny of behavioral interactions (Aggression and Dominance) in beagle and terrier dogs and their hybrids.

Principal Investigator: Walter C. Stanley

Other Investigators: Gerson Grosfeld, Robert E. Stetson, Stephen N. Lee

Cooperating Units: None

Man Years

Total:	1.46
Professional:	.20
Other:	1.26

Project Description:

Objectives: To observe, record, and quantify stable and changing patterns of behavioral interactions (aggression, dominance, etc.) as they naturally occur in intact litters of purebred beagles and wirehaired fox terriers and their reciprocal crosses. The breeds were selected because they differ markedly in their genetic predisposition to fight and kill. Terriers have been selectively bred for fighting and killing whereas beagles are comparatively nonaggressive dogs.

Methods Employed: Eight 20 x 80 ft. outdoor pens have been constructed to house intact litters, of which four to six will be used at any one time for the study. Intact litters will be observed from birth through 1 - 2 years of age, utilizing primarily time-lapse, closed circuit TV recording of samples of entire litter behavior 1-5 days per week.

Major Findings:

Experimental Caretaking: Preliminary work has been carried out

on two 4-dog and two 6-dog beagle litters. This work indicated the need for a completely experimental caretaking procedure, eliminating aversive stimulation which generates timid and withdrawal behavior in dogs. An experimental caretaking procedure was therefore instituted which provides for the expression of natural canine behavior unaltered by coercive intervention typical of normal caretaking. Observations made each day (before, during, and after experimental caretaking) indicated that a 20 x 80 ft. pen is large enough for the free expression of social and individual behavior typical of small-sized dogs such as beagles. Initially, a break in the routine of experimental caretaking temporarily suppressed the dogs' social behavior. Two-three months after institution of the experimental caretaking, breaks in the procedure tended not to produce the temporary behavior suppression.

Preliminary Analysis: Findings on experimental caretaking have led to the following formulation of overall method: first, to evaluate procedures for accumulating ethograms on individual dogs and on litters with a pilot study on one litter each of beagles and terriers; second, to conduct a study on four litters, one of each pure breed and one for each of the two crosses; and third, to replicate the study on four additional litters. Considerable planning remains. Data accumulation on the first replication of four litters probably will not start until late in summer or early fall.

Significance: Deviant and violent expression of aggression and dominance are characteristic of some severe behavioral disturbances in people. The ontogenic study of such behavior in two genetically different breeds of domestic dog, should provide new approaches to the conceptualization of deviant violent behavior. The discovery of an heuristic animal model for use in behavioral, pharmacological and in brain and behavior studies is a distinct possibility.

Proposed Course: To terminate the project after eight litters have been studied, two each of beagles, terriers, beagle X terrier crosses, and terrier X beagle crosses.

Honors and Awards: None

Publications: None

Serial No. M-LBEB-BS-2
1. Laboratory of Brain Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Social velocity

Previous Serial Number: Same

Principal Investigator: John B. Calhoun

Other Investigator: Halsey M. Marsden

Cooperating Units: Office of the Director, IR, NINDS (H. M. Marsden assigned to SOBS from NINDS. During the course of the study prior to FY '74 and during FY '74 he has continued to collaborate in the analysis)

Man Years

Total:	1.95
Professional:	0.35
Other:	1.60

Project Description:

Objectives: To measure social velocity in groups of rodents and to determine how the variability of velocity among members of a group relates to social organization and individual welfare.

Methods Employed: As detailed in prior reports. It essentially involves obtaining quantitative measures of the frequency with which each individual in a group is active or alert in those portions of the environment where social status interactions more often occur. Such measures provide an estimate of social velocity.

Major Findings: In optimum sized groups (of size N_o) after the animals are ranked according to their velocity, the difference in velocity between any two adjacently ranked individuals is a constant, equivalent to the velocity of the lowest ranked individual. When the group size exceeds the optimum (e.g. when it reaches $2N_o$) the total group divides into two subgroups. One

subgroup maintains essential normality with regard to degree and variability of velocity. All members of the other subgroup have velocities lower than the lowest velocity individual in the more normal subgroup. Despite genetic identity of the inbred strains utilized, the members of the reduced velocity subgroup have, through maturation in a crowded social context, become as distinct from the normal subgroup as are two biological (genetic) species. Such low velocity "behavioral species" are further characterized by fragmented, deviant behavior, and so little difference in velocity characterizes adjacently velocity ranked individuals that it is highly unlikely that individuals can recognize associates as unique individuals. This finding represents the first major conceptual breakthrough with regard to social velocity since we first described this concept in 1963!

Significance: The concept of social velocity is becoming more and more useful in understanding the dynamics of organization of social groups, particularly with reference to crowding. It provides a methodology for determining the optimum group size with respect to any combination of hereditary constitution, environmental structure or modifications of physiology or behavior induced by pharmacological agents or CNS lesions.

Proposed Course of Project: Further studies of social velocity will be conducted as special aspects of the continuation of investigations listed under BS-3 and BS-8.

Honors and Awards:

Presented an invited paper, "The Effect of Crowding on Social Velocity," at the 1973 meeting of the American Psychiatric Association as a part of the symposium on "Social Psychiatric Implications of Population Control."

Publications: None.

Serial No. M-LBEB-BS-3
1. Laboratory of Brain Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The overliving of a mouse population

Previous Serial Number: Same

Principal Investigator: John B. Calhoun

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.80
Professional:	0.25
Other:	1.55

Project Description:

Objectives: To gain an understanding of how the overcrowding that develops through several generations in an environment replete with resources culminates in the behavioral disorganization of all members.

Methods Employed: As described in prior annual reports on this project. See below for other details under "Proposed Course of Project."

Major Findings: There are no new results from analyses. Most of the effort on this project during this fiscal year has been directed at completing the computer tape file that will permit adequate analysis.

Proposed Course of Project: Utilizing all our insights from past studies we have designed a larger and more adequately designed physical habitat in which to reinitiate a similar study. Now knowing much more about the time of population events, and which changes in behavior and social organization are particularly important, we are planning the course of the next study to make observations that will more effectively contribute to the understanding of crowding related phenomena.

It is our hope that before initiation of this study -- which will last for a minimum of three years -- that we will be able to make collaborative arrangements with other research groups to participate in it. In particular, we know that this proposed study will be less than fully adequate unless it includes efforts which will help to gain an understanding of the changes occurring in catecholamine and vitamin A metabolism, and unless more detailed studies are made of the breakdown of maternal behavior and of the inadequacies of mice which are reared in a complex environment without adequate mothering.

Significance for Biomedical Research and the Program of the Institute: Insights from the analyses of studies conducted under this project to date indicate that the disorganization of behavior under conditions of overcrowding have their beginning in processes of social interaction in mice which seem to be related to those associated with child abuse on the human level. Early behavioral disorganization then becomes potentiated through an increase in the rate of storage of vitamin A. It appears that we have developed a research strategy regarding phenomena that are important on the human level.

Honors and Awards: See last year's report.

Publications:

Calhoun, J. B.: Plight of the Ik and Kaiadilt is seen as a chilling possible end for man. In Rebelsky, Freda and Dorman, Lynn (Eds.): Child Development and Behavior. Alfred A. Knopf, New York, 1973: pp. 328-334.

Calhoun, J. B.: From mice to men. Transaction and Studies of the College of Physicians of Philadelphia, 41(2): 92-118, 1973.

Calhoun, J. B.: Population density and social pathology. In Wilson, E. O. (Ed.): Ecology, Evolution, and Population Biology. W. H. Freeman and Co., 1974: pp. 109-116.

Serial No. M-LBEB-BS-8
1. Laboratory of Brain Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Environmental modification of individual behavior and social organization

Principal Investigator: John B. Calhoun

Other Investigators: Leonard E. Olson, Virginia Polytechnic Institute

Cooperating Unit: Department of Physics, Virginia Polytechnic Institute

Man Years

Total:	1.80
Professional:	0.25
Other:	1.55

Project Description:

Objectives: To design behaviorally meaningful physical environments for rodents (rats and mice) capable of sustaining groups or populations, and to determine the character of the utilization of time and space within these settings, particularly with reference to individual behavior and social organization.

Methods Employed: These fall under two categories: (1) the environmental design process itself; and (2) behavior recording technology. With regard to environmental design, considerable effort and thought have been devoted to constructing models of environments proposed for the conduct of future studies. These models prove very useful in pursuing a simulation of the conduct of each study. In the designing process emphasis is placed on developing a semantics of the environment in which the structure and arrangement of subdivisions of it carry unambiguous messages as to the behaviors appropriate to each subspace. With regard to recording technology, our automated data acquisition is in its second stage of development. It permits recording the identity of each animal subject, its place, time and direction of travel at all times through the history of a group. In addition, the new system permits online control of cooperative

behavior; that is to say, programs can be written that define which members of a group can engage in cooperative behavior in a drinking situtation.

Major Findings: Remarks here will be confined to currently completed analyses of behavior in a compartmentalized environment with regard to its time-space configuration. In each compartment two kinds of behavior occur: (1) those related to an opportunity provided for fulfilling a basic need (such as related to eating, drinking or sleeping); and (2) other individual or social behaviors which the experimental subjects elect to express there. Analysis of the durations of stay within compartments reveals that Type 1 and Type 2 behaviors become bound together with reference to their initiation and termination. In addition, there are a set of short duration behaviors in each compartment associated with exploration and social bonding. A unique exponential equation describes number of behaviors as a function of their duration, for each type of behavior in each compartment by every individual. This permits behavior typing of every individual in terms of time-space ethograms based on these equations. L. E. Olson is completing a doctoral thesis related to this project. It encompasses a description of the data acquisition system, and a presentation of a wide range of individual and social behavioral phenomena capable of examination by this system. Mostly they are phenomena that cannot be recorded by visual observation.

Proposed Course of the Project: It is anticipated that a redesigned habitat, utilizing the automated data acquisition system (DAS) will be completed in time to initiate during the fiscal year 75 the first of a series of studies. The first study will involve an examination of social entropy and negentropy based on the "Maxwell's Demon" model which led through Szilard to Shannon's elaboration of information theory. In practice what will be done is to monitor the social velocities of two non-communicating groups of rats. Periodically the high velocity rat from one group will be placed into the other group, and at the same time a reverse direction shift will be made of a lowest velocity rat. At the present time there seems to be no rational basis for predicting the outcome. Three possibilities exist: (1) both groups will increase in entropy; (2) both will increase in negentropy (synergy); or (3) one will increase in entropy and the other in negentropy.

Significance: The precision and quantity of the DAS data opens a vista of developing a social physics in which more, and more precise, insights about behavior can be gained thrcugh it than can be gained from visual observation. The kinds of insights will be different, and hopefully the two kinds of approaches can eventually be integrated.

Honors and Awards:

Calhoun has been invited to prepare the opening, theme-setting address of a conference on "The Evolution of Intelligent Environments" to be held during the spring of 1975. This conference is cosponsored by the Schools of Architecture of the Massachusetts Institute of Technology and the University of Texas.

Publications: None.

Serial No. M-LBEB-BS-9
1. Laboratory of Brain Evolution and Behavior
2. Section on Behavioral Systems
3. Poolesville, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Population, space and mental health

Previous Serial Number: Same

Principal Investigator: John B. Calhoun

Other Investigators: Garrett A. Bagley, David B. McCarn (NLM), Edith G. Calhoun (NLM)

Cooperating Unit: National Library of Medicine

Man Years

Total:	1.10
Professional:	0.45
Other:	0.65

Project Description:

Objectives: To determine those means whereby the mental health and wellbeing of human beings can be holistically understood in terms of evolution, environmental design, concepts, and systems of organization.

Methods Employed: The major line of approach involves developing systems of identifying the more important literature, producing creative abstracts, constructing a network of concept relationships, and producing permutated indexes. A secondary, but probably equally important methodology, involves detailed dialogue with others, who share the perspective that the human species has entered a critical phase shift in evolution in which the decisions made and the processes initiated will determine the future course for a long time into the future.

Major Findings: In the area of information processing they are threefold: (1) We have refined methodology of preparing permutated indexes which enables and permits many kinds of "random walk" or directed search exploration of the literature indexed. This refinement was made during the course of preparing an overview of the preconference papers for the Rome 1973 Special

Conference on Futures Research. (2) On the basis of 6,000 concept associations we have been able to continue with the construction of a concept network that should prove useful in contributing to the development of computer formulation of both creative and consensual texts. (3) A novel matrix search strategy was developed and applied to the MEDLARS-MEDLINE systems and data base of the National Library of Medicine. Utilizing it, 480 literature searches were run. They culminated in the preparation of a special bibliography on the psychosocial consequences of population and environment, which provides the basis for defining the scope of this broad, complex field with respect to the emphases present in the research publications of behavioral scientists.

Proposed Course: The above mentioned bibliography will be utilized to prepare an evaluation of the scope of concerns it encompasses.

Honors and Awards:

Invited participant in the Rome 1973 Special Conference on Futures Research.

Gave a three minute filmed commentary on the population problem to be utilized to introduce a film on population being prepared by Roberto Rossellini at the request of the United Nations for presentation in connection with the United Nations Population Year and the United Nations Conference on this subject in Bucharest, Rumania in August 1974.

Participated in a dialogue on Human Reality sponsored by the Menil Foundation, Houston, Texas, December 6 - 9, 1973. The other four members of the dialogue group were: Dr. Jonas Salk of the Salk Foundation, Dr. Joel Elkes of the Department of Psychiatry, Johns Hopkins University, Dr. Giorgio La Pira, former mayor of Florence, Italy, and Archbishop Helder Camara of Recife, Brazil.

Invited participant at Oxford, England, in the Sunday Times-United Nations Population Fund Conference on "Exploding Cities" as a part of the U. N. Population Year - 1974. This conference brought together 50 publishers or journalists from leading world newspapers and magazines with 50 behavioral scientists concerned with population or the urban physical and social environment.

Gave an eleven minute filmed commentary (to be edited down to six minutes) for the West German and Italy National

television companies on the implication of our experimental studies of animal populations. This will be part of a 45-minute documentary prepared in conjunction with the U. N. Population Fund office. The three others preparing commentaries are Dr. Freedman of Columbia University, and Drs. Barney Berelson, and Paul Ehrlich.

Publications:

Calhoun, J. B.: Environmental design research and monitoring from an evolutionary perspective. Man-Environment Systems, 4(1): 3-30, 1974.

Calhoun, J. B.: Population problems: Two disciplines agree. In Fleck, Stephen, (Ed.): Humane Reproduction. Group for the Advancement of Psychiatry. 1973. Vol. VIII, Report No. 86: pp. 431-440.

Calhoun, J. B., (Ed.): Psychosocial Consequences of Population and Environment: An Experimental Bibliography, Phase I. U. S. Dept of Health, Education and Welfare (National Library of Medicine and National Institute of Mental Health). March 1974. 326 pp.

Serial No. M-CM-DN-1
1. Laboratory of Cerebral Metabolism
2. Section on Developmental Neurochemistry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The mechanism of action of thyroxine and its relation to cerebral metabolism

Previous Serial Number: Same

Principal Investigator: Louis Sokoloff, M.D.

Other Investigators: Patricia A. Roberts, Barbara A. Jant, Elaine E. Kaufman, Gilman D. Grave, R. J. S. Duncan, Glenna G. Fitzgerald, Ellen G. Archer, John E. Kline and Esther L. Lewis

Cooperating Units: None

Man Years:

Total:	5.5
Professional:	3.5
Other:	2.0

Project Description:

Objectives: The ultimate goal of this project is the elucidation of the role of the thyroid hormone in the maturation and development of the brain and the influence of the hormone in the regulation of cerebral metabolic processes. A necessary intermediate objective is the determination of the molecular mechanisms of the physiological and biochemical actions of the thyroid hormone. Earlier work done previously in this project demonstrated a stimulating action of thyroid hormones on protein biosynthesis. The mechanism of action of thyroxine still remains the primary focus of the project.

Methods Employed: The effects of thyroid hormones on radioactive amino acid incorporation into protein are examined in cell-free protein synthesizing systems from various mammalian tissues, such as liver, brain, reticulocytes, etc. Studies are occasionally carried out in vivo, in which the uptake of amino acids into protein in various tissues is measured in the intact animal or the effects of experimentally induced thyroid disease on various metabolic activities are examined. A wide variety of enzyme purification and assay procedures are also employed. Enzymes are purified from brain or liver homogenates by detergent treatment, salt fractionation, gel adsorption and filtration, ion exchange chromatography, etc. Assays of enzyme activities utilize chemical analyses, spectrophotometric techniques, and Geiger-Mueller and liquid scintillation radioactivity measurements.

Major Findings: The major accomplishment of this project thus far is the demonstration for the first time that thyroxine stimulates protein biosynthesis and that this effect is probably primary to most of the generally recognized physiological actions of the thyroid hormones. Thyroxine, administered in vivo or in vitro, stimulates amino acid incorporation into protein in cell-free rat liver preparations. Conversely, hypothyroidism induced by surgical thyroidectomy, results in a reduction in the rate of amino acid incorporation into protein in similar preparations, indicating that the thyroid effect on protein biosynthesis is tonically active and physiological.

The biochemical mechanism of the thyroxine stimulation of protein biosynthesis has been studied in considerable detail in completely in vitro systems. These studies have been carried out over a number of years and have demonstrated that the thyroxine stimulation of protein synthesis is exerted by two mechanisms. There is a direct effect of the translational activity of ribosomes leading to accelerated completion and release of newly synthesized polypeptide chains followed, in vivo only, by an increased synthesis and accumulation of ribosomes, i.e., a transcriptional effect. The transcriptional effect is not a direct action of the hormone but a cellular response or compensation for some earlier effect. The early effect is the one most likely to lead to the elucidation of the primary mechanism of action of the thyroid hormones and is the one which has been of greatest interest to the Section.

The early translational effect has also, however, been found to be secondary to some early direct energy-dependent reaction between thyroxine and the mitochondrial components of the cell. Some still unidentified product of this reaction is found which acts on the ribosomes to stimulate their translational activity. Work has been continuing to isolate, purify, and identify this active factor. It is now known to be a small, dialyzable, heat-stable, acid-labile, anionic, organic, probably non-lipid substance. Continued work on it has increased the knowledge of its properties and narrowed the range of possibilities, but its exact identity still eludes us. This research has already led to the explanation of the change in sensitivity to thyroid hormones which occurs in brain during development. The brain responds to thyroid hormones in a manner similar to other organs during the period of its development and maturation. Once mature, however, it loses its responsivity to the hormones. This change can be seen in the effects of thyroid hormones on oxidative metabolism, protein synthesis, etc. One outcome of this project has been to show that the loss of sensitivity of the brain to thyroxine with development is the result of some change in the mitochondria so that they lose the ability to react with thyroxine and form the active factor responsible for the stimulation of protein synthesis.

Recent conflicting claims in the literature that thyroxine in vitro can stimulate protein synthesis directly without involvement of mitochondria have been critically examined in this Laboratory during this past year. The results clearly and decisively prove that these contradictory findings were the outcome of an artifact arising from the uncritical use of uniformly labeled amino acids of very high specific activity. Our results also provide a basis for careful reevaluation of many published studies in which this artifact could have been operating. The results of our recent studies are in the process of publication.

Evidence has been accumulating that thyroid hormones may also have important effects on the transport of ions into and out of cells. Experiments have been carried out in this Laboratory on thd effects of thyroxine on the various microsomal ATPases in rat liver and brain cells. Among them is a Na^+,K^+-stimulated ATPase which is intimately involved in the active transport of Na^+ and K^+ between cells and their external environment.

Thyroxine *in vitro* has been found to inhibit the overall microsomal ATPase activity. There is no specificity for the various ATPases. In liver microsomes, the Mg^{++}-, the Ca^{++}-, and the K^{+},Na^{+}-stimulated ATPases are all inhibited equally by thyroxine, and qualitatively similar but quantitatively greater effects occur with isolated liver plasma membranes and brain microsomes. These results provide additional evidence that thyroid hormones have a role in the regulation of intracellular ion concentrations. This would in turn have major effects on a vast variety of intracellular metabolic processes. In order to ascertain whether these effects found *in vitro* have physiological significance, the effects of experimental thyroid disease have been investigated. Hyperthyroidism leads in liver but not in mature brain to an enhancement of the microsomal ATPases, particularly the Ca^{2+}- and Mg^{2+}-activated species. These effects are in the opposite direction from those seen with the hormone *in vitro*. These opposing effects can be reconciled by the hypothesis that the direct effect of thyroid hormones is to inhibit the ion-dependent microsomal ATPases, but *in vivo* the cells compensate for this inhibition by producing more enzyme. Recently completed experiments indicate, in fact, that the increased ATPase activities in liver in hyperthyroidism are indirect consequences of the disease. The increased activities develop slowly not becoming significant until at least 18 hours after a dose of hormone. Furthermore, starvation alone produces the same effect and blocks this effect of thyroid hormones. Since hyperthyroidism, by elevating energy metabolism, produces a relative nutritional deficiency it is conceivable that the increased ATPase activities are caused by the relative nutritional deficiency resulting from the hypermetabolic state. This possibility of a close link between thyroid effects and nutrition will be investigated further.

The thyroid hormones promote the structural, functional, and biochemical maturation of the brain. In addition to the studies of the mechanism of action of the thyroid hormones, the Section has also been studying the nature of the biochemical changes which occur in the brain with maturation. A prominent developmental change in the brain is a rise in the level of mitochondrial enzymes involved in oxygen consumption and energy metabolism of the brain. One of these, D-ß-hydroxybutyric dehydrogenase (BDH), was found in this Laboratory to rise with brain development just like other mitochondrial enzymes but,

unlike these others, such as cytochrome oxidase and succinoxidase, to decline again gradually following weaning. Evidence has been obtained that the changes in the level of this enzyme in brain are, at least, in part related to nutritional factors. It has also been found recently that hyperthyroidism shifts the developmental pattern in this enzyme to an earlier age (e.g., it causes a more rapid and earlier rise in the enzyme level in the brain, an earlier achievement of its peak level, and an earlier decline). In the course of these studies an interesting observation was made which might be a valuable model for the elucidation of the mechanism of action of thyroxine in mitochondria. Physiologically active thyroid hormones and their analogues were found in vitro to inhibit BDH activity at extremely low concentrations. The inhibition was found to represent a competitive inhibition by the hormone of the binding of the normal substrates to the enzyme. The enzyme is a membrane-bound enzyme in mitochondria. After considerable effort it has been solubilized and partially purified several-fold from both brain and liver. The highest purification thus far achieved has been about 30-fold, but the finding in the last 2 weeks of beneficial effects of glycerol on the enzyme stability and activity provide hope of markedly better purifications. The purified enzyme is to be used to study the mechanisms of the thyroxine-inhibition as well as the mechanisms of the regulation of the enzyme's activity through all its changes during postnatal maturation of the brain. It has already been found with the partially purified enzyme that the enzyme requires a phospholipid cofactor normally present in mitochondria for its activity. This cofactor has been identified as a species of lecithin which can be partially substituted for in vitro by commercial beef heart or egg lecithin. The natural cofactor has been partly purified, and progress has been made to identify the nature of its fatty acid composition. The dioleoyl-lecithin is the most active species examined, but the exact identity is still under investigation.

Significance to Bio-medical Research and the Program of the Institute: A unique feature of the cerebral metabolism is its apparent lack of response to high circulating levels of thyroid hormone. An understanding of the basis of this unique behavior may reveal information concerning the metabolism of the brain in health and disease. Also, the mechanism of action of thyroxine has been under investigation for many decades, but thus far a satisfactory explanation of the mechanism by which it

increases metabolic rate, stimulates metamorphosis and growth, or causes the many disturbances in body physiology and biochemistry in thyroid disease has eluded investigators. The solution of this problem could then lead to fundamental advances not only in the understanding of how this important hormone regulates bodily functions but also of the nature of bodily processes on which it acts. The clarification of the relationship between its mechanism of action and its effects on development of the brain might also lead to a better understanding of the many biochemical processes underlying the maturation of the brain.

Proposed Course of Project: The project will be continued essentially on the course already indicated. The major avenues to be pursued are: (1) the attempts to isolate, purify, and identify the product of the thyroxine-mitochondrial reaction responsible for the stimulation of protein biosynthesis; (2) further studies on the elucidation of the mechanism of the stimulation of the protein biosynthetic processes; (3) the further study of the thyroxine effects on ion-dependent ATPases; (4) further studies of the biochemical differences between mature and immature brain and the role of thyroxine in the biochemical changes leading to maturation and development of the brain; (5) purification of brain D-β-hydroxybutyric dehydrogenase; and (6) studies of the mechanism of the inhibition of D-β-hydroxybutyric dehydrogenase by thyroxine.

Honors and Awards: None

Publications:

Sokoloff, L., and Raskin, N. H.: Chapter 96. Thyroid Disorders. In Shy, M., Appel, S., and Goldensohn, E. (Eds.). The Cellular and Molecular Basis of Neurological Disease. New York, Lea & Febiger, 1969 (in press).

Sokoloff, L., and Kennedy, C.: The action of thyroid hormones and their influence on brain development and function. In Gaull, G. E. (Ed.). Biology of Brain Dysfunction. New York, Plenum Publishing Corporation, 1973, pp. 295-332, Vol. 2.

Sokoloff, L.: Metabolism of ketone bodies by the brain. Ann. Rev. Med. 24: 271-280, 1973.

Sokoloff, L.: Introduction to regulatory biochemistry of neural tissues. In Schmidt, F. O. (Ed.). The Neurosciences, Third Study Program. Cambridge, Mass., MIT Press, 1973, pp. 809-811.

Sokoloff, L.: Changes in enzyme activities in neural tissues with maturation and development of the nervous system. In Schmidt, F. O. (Ed.). The Neurosciences, Third Study Program. Cambridge, Mass., MIT Press, 1973, pp. 885-898.

Fellous, A., Francon, J., Nunez, J., and Sokoloff, L.: Protein synthesis by highly aggregated and purified polysomes from young and adult rat brain. J. Neurochem. 21: 211-222, 1973.

Pommier, J., Sokoloff, L., and Nunez, J.: Enzymatic iodination of protein. Kinetics of iodine formation and protein iodination catalyzed by horse-radish peroxidase. Eur. J. Biochem. 38: 497-506, 1973.

Fitzgerald, G. G., Kaufman, E. E., Sokoloff, L., and Shein, H.: D(-)-β-hydroxybutyrate dehydrogenase activity in cloned cell lines of glial and neuronal origin. J. Neurochem., 1973 (in press).

Sokoloff, L., and Roberts, P. A.: Artifacts in studies of protein synthesis with radioactive amino acids. Invalidity of alleged stimulation of protein synthesis by thyroxine in vitro in absence of mitochondria. J. Biol. Chem., 1974 (in press).

Serial No. M-CM-DN-2
1. Laboratory of Cerebral Metabolism
2. Section on Developmental Neurochemistry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on regional cerebral circulation and metabolism

Previous Serial Number: Same

Principal Investigator: Charles Kennedy, M.D.

Other Investigators: Louis Sokoloff, Michel H. Des Rosiers, Jane W. Jehle, John E. Kline and Esther L. Lewis

Cooperating Units: Martin Reivich, M.D., Associate Professor, Department of Neurology University of Pennsylvania Philadelphia, Pennsylvania

Clifford S. Patlak, Ph.D., Theoretical Statistics and Mathematics Branch, NIMH

Man Years:

Total:	3:00
Professional:	1.50
Other:	1.50

Project Description:

Objectives:

1. To continue to elucidate the patterns of development of circulation and energy metabolism in various structural components of the developing mammalian brain.

2. To complete the development and validation of a method for measurement of local glucose utilization of the brain in vivo and to establish normal values in various laboratory animals.

3. To demonstrate the usefulness of the method for measurement of local cerebral glucose utilization for mapping functional pathways in the brain.

4. To study states of altered mental activity (such as anxiety, rage, sleep, thirst) and drug-induced alterations of neural function in an effort to localize structures related to such behavior.

Methods Employed:

The method for local cerebral glucose utilization involves the use of the radioactive tracer, ^{14}C-deoxyglucose, an analogue of glucose, which shares with glucose a common transport system in its exchange between blood and brain. Its rate of accumulation in brain tissue following an intravenous infusion is measured by quantitative autoradiography. A knowledge of the concentration of both this tracer (^{14}C-deoxyglucose) and glucose in arterial blood over the time period of the experiment plus a knowledge of a number of kinetic constants permits a calculation of the rate of glucose utilization in a given structure of the brain.

Major Findings:

1. The theoretical formulation by which calculations are made has been expanded and it now eliminates the need for obtaining several constants of the hexokinase reaction for glucose and deoxyglucose in vivo. While these might have been determined by a series of elaborate, separate physiologic studies, the new formulation expresses these constants as the product of two ratios and hence they appear as a new "lumped" constant. This can be determined relatively easily for any species of laboratory animal by measurements of extraction ratios of glucose and deoxyglucose. At present we are using that obtained by our collaborator, Dr. Marvin Reivich, who found this to be approximately 1.0.

Remaining in the revised equation are values for the rate constants for the exchange of deoxyglucose between plasma and brain. In the previous year these had been determined experimentally for the non-metabolized glucose analogue, 3-O-methyl glucose. Although the values obtained for this analogue could be considered close approximations to those of deoxyglucose, a refinement of the method required that they be determined for deoxyglucose itself. Therefore the necessary more extensive studies were carried out and the data obtained submitted to computational analysis (M lab program) by our collaborator, Dr. Clifford Patlak. The values obtained are currently being used in our first reports of normal values for local cerebral glucose utilization, but by extending the series of studies for the estimation of these rate constants we expect to increase their reliability and thereby further reduce a small source of error in the method.

In practice we have established optimal conditions by infusing the tracer as a single pulse and by monitoring the tracer concentration for 30 minutes before removal of the brain. With the laborious chore of the long and complex calculations having been immensely expedited by our newly-acquired laboratory computer (Hewlett-Packard 30) programmed for our particular equation, we have completed measurements in 23 structures in a series of awake, conscious rats. An average of the values obtained is close to that which might have been predicted from a knowledge of values for overall glucose utilization in other species. The individual values for the various component structures were distributed over a wide range and in a bimodal distribution with values for white matter being uniformly low and separate from the higher, heterogeneous values in gray matter. The structures related to hearing were found to command the highest rates of glucose utilization.

The pattern in which values for local glucose utilization are distributed in the various component cerebral structures is very similar to that which has been found for blood flow. The similarity was particularly striking when the data were subjected to a regression analysis, the co-efficient of correlation approaching unity (0.96).

By way of validation of the method, we have examined the effect of light thiopental anesthesia. As had been predicted

there was a consistent reduction of values in both white and gray structures. Measurements of local cerebral blood flow in a similar group of anesthetized rats yielded values which were reduced from normal by approximately the same percentage. This series of studies of local glucose utilization and local blood flow not only served to validate our new method but provided the first direct evidence for a coupling of blood flow and metabolic rate in brain. To demonstrate that our method could measure glucose utilization independently of blood flow, we designed a series of experiments in which we predicted that the two variables would be dissociated. The administration of 5% carbon dioxide in the inspired air could be expected to accomplish this by acting to increase blood flow but to be without effect on glucose utilization. This work is still in progress but in the studies completed to date the expected dissociation has, indeed, been demonstrated.

Previous work has indicated that alterations in functional activity in the nervous system are accompanied by corresponding changes in metabolic rate in the particular structures involved in such functional activity. To demonstrate the usefulness of our method in determining loci for any given neural function, we have made measurements of glucose utilization in animals in which brain function has been altered. In most of these the alteration has been asymmetrical. Inasmuch as the normal, resting, conscious animal has been found to have negligible differences in rate of glucose utilization of individual structures between right and left sides, the finding of large differences can be attributed to the specific (asymmetrical) functional alteration. Below are listed some of the changes which have been found:

1. The removal of one eye in the rat resulted in contralateral reduction in the glucose utilization in the lateral geniculate body, superior colliculus, and visual cortex. The findings are in keeping with the neuroanatomic relationships in the rat in which 85% of the fibers of the optic nerve cross and in which the brain stem dominates in the mediation of visual responses in this species.

2. The removal of one eye in the rhesus monkey and subsequent measurement of local glucose consumption indicated the presence of two pairs of cortical regions with marked right-left

differences in glucose consumption. The findings suggested that these regions unlike the rest of the visual cortex received stimuli from only one eye. In the case of one pair of these monocular cortical loci the corresponding eye was ipsilateral while in the other it was contralateral. We have some reason to believe that the pair of ipsilateral representation corresponds to the blind spot (the region occupied by the optic nerve) of the retina.

3. Bilateral visual stimulation of the rhesus monkey by a moving geometric pattern causes increases in glucose utilization in mosaic or columnar pattern in the striate cortex.

4. Local (epileptic) seizure activity induced by an intracortical injection of penicillin into a small region of the primary motor cortex of the rhesus monkey was found to result in an increase in the glucose utilization in several discrete regions of the motor and sensory systems of the brain. These included (a) a limited region of the cortex; (b) small regions within the putamen, globus pallidus and caudate nucleus and a discrete part of the thalamus judged to be parts of the nuclei ventralis posterolateralis and ventralis posteromedialis.

5. A monkey engaged in continuous unilateral hand and arm movements had higher values for glucose utilization in the contralateral regions of the motor strip of the cortex and in a number of regions of the basal ganglia than were found on the corresponding ipsilateral regions of these structures.

Significance to Biomedical Research and the Program of the Institute: The successful development of the method for local glucose utilization in brain has particular significance. For years there has been a hypothesis that blood flow to various regions of the brain is regulated according to the metabolic demand. The method now demonstrates directly and unequivocally that the hypothesis is indeed correct, and as a result much greater significance can now be attributed to the body of literature reporting on changes in local blood in the brain under a variety of physiologic conditions. The method opens new important areas of investigation of interest to workers in other disciplines such as neuropharmacology, neurophysiology and neuroendocrinology. It will provide an extremely useful tool in establishing the loci and neuronal centers involved in such

functional activity as sleep, specific motor and sensory function, and various affective states, to mention only a few.

It also will be important in establishing the locus of action of drugs and will provide a means of mapping the sequence of brain centers which are activated following electrical stimulation. This will be particularly useful for the study of epilepsy in animal models.

For those concerned with brain growth and conditions essential to its optimal development the method promises to be a powerful experimental tool. During maturation glucose is not only an essential substrate to provide energy for functional activity but it is required for the synthesis of nucleic and amino acids as well as phospholipids. It has been shown by others that normal blood levels of glucose in infancy do not necessarily reflect a sufficiency at a cellular level. Furthermore, the young, especially the newborn, may have no behavioral change in the face of known deficiency in the blood. Hence it becomes important to determine experimentally the conditions (with respect to blood glucose levels, ketone body levels, oxygen availability, blood flow, etc.) which are optimal for normal brain growth. In such studies a method for cerebral glucose utilization could be expected to be invaluable.

Proposed Course of Project: With the finalization of the development of the method for local glucose utilization in brain we will return to the problems of the energy requirements of the brain during development and examine the relationship between cerebral glucose and D(-)-β-hydroxybutyrate consumption during postnatal maturation. The new method for local glucose utilization will also be applied to various states of altered mental function induced by disease, drugs, or physiological stimulation.

Honors and Awards:

Honored Guest, International Symposium on Cerebral Blood Flow, Philadelphia, Pennsylvania, June 6-9, 1973.

Publications:

Eckman, W. W., Phair, R. D., Fenstermacher, J. D., and Sokoloff, L.: The influence of capillary permeability limitations on the measurement of regional cerebral blood flow. 6th International Symposium on Cerebral Circulation and Metabolism, Phila., Pa., 1973 (in press).

Des Rosiers, M., Grave, G. D., Kupferberg, H. S., and Kennedy, C.: Effects of diphenylhydantoin on local cerebral blood flow. 6th International Symposium on Cerebral Circulation and Metabolism, Phila., Pa., 1973 (in press).

Sokoloff, L.: Aging, Senescence, and Dementia. In Paulson, O. B. and Arbus, L. (Eds.) Proceedings of Symposium on "Cerebral Blood Flow and Cerebral Metabolism", 1973, Paris, France (in press).

Kennedy, C., Des Rosiers, M., Reivich, M., and Sokoloff, L.: Mapping of functional pathways in brain by autoradiographic survey of local cerebral metabolism. In Transactions of American Neurological Association, 1974 (in press).

Serial No. M-CM-DN-3
1. Laboratory of Cerebral Metabolism
2. Section on Developmental Neurochemistry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical bases of alcohol addiction

Previous Serial Number: M-CS-CM-11

Principal Investigator: Louis Sokoloff, M.D.

Cooperating Units: Neil H. Raskin, M.D., Department of Neurology, University of California Medical Center, San Francisco, Calif.

Other Investigators: None

Man Years:

Total:	0.25
Professional:	0.25
Other:	0

Project Description:

Objectives: This project is directed at the acquisition of knowledge of the biochemical changes associated with chronic alcohol ingestion and the integration of such knowledge into a framework which it is hoped will provide a basis for the understanding of the mechanisms underlying alcohol addiction and the withdrawal effects.

Methods Employed: The enzymes associated with ethanol metabolism and that of its products are assayed in brain, liver, and other tissues of normal rats and rats fed various amounts of ethanol for various periods. Classical methods of enzymology will be employed. The results of the enzyme assays of enzyme activity in brain are correlated with the results of behavioral tests of alcohol tolerance or alcohol-withdrawal symptoms.

Major Findings: This project has been a collaboration with Dr. Neil H. Raskin, a previous Research Associate in this Section, and presently an Associate Professor in the Department of Neurology, University of California Medical Center, San Francisco, California. This project had previously demonstrated by means of a new assay technique the presence of alcohol dehydrogenase, the enzyme responsible for the metabolism of ethanol, in the brain. Previous efforts by other investigators to find the enzyme in brain had been unsuccessful because of inadequately sensitive and specific methods. The kinetic properties and the responsivity to specific inhibitors of the brain enzyme have been examined and compared to those of the liver enzyme and found to be almost identical. A tissue survey of the alcohol dehydrogenase has revealed its presence in all tissues which exhibit pathological changes with chronic excessive alcohol ingestion. In studies in rats it was found that the brain enzyme activity rises about 50% with chronic alcohol ingestion. This is in contrast to the liver enzyme which remains unchanged. Behavioral tests of tolerance to alcohol have now been carried out, and the results show a very close temporal correlation between the changes in alcohol dehydrogenase activity in brain and in the level of tolerance. Both the tolerance and brain enzyme activity rise very slowly after the beginning of alcohol feeding, reaching their new steady state levels at about two weeks. Both changes, however, fall rapidly to normal within 48 hours after alcohol withdrawal. No symptoms of alcohol withdrawal are seen. This is the first evidence of a specific enzyme change in the brain with alcohol intake and suggests a possible relationship to the development of tolerance to ethanol and/or a biochemical basis for the development of CNS symptoms following withdrawal from alcohol after prolonged ingestion.

Significance to Bio-medical Research and the Program of the Institute: Numerous drugs and chemical agents cause addiction which results in withdrawal symptoms when their use is discontinued. Alcohol is one of the most common. Although it is clear that some biochemical change must have occurred, none yet has been found which could satisfactorily explain the phenomenon. Elucidation of the biochemical mechanism of alcohol addiction might not only serve as a model for other types of addiction but might also lead to a more rational approach to therapy.

Proposed Course of Project: This project will be temporarily discontinued because of the lack of adequate personnel.

Honors and Awards: None

Publications:

Raskin, N. H., and Sokoloff, L.: Changes in brain alcohol dehydrogenase activity during ethanol ingestion and withdrawal. J. Neurochem., 1973 (in press).

Serial No. M-CM-DN-4
1. Laboratory of Cerebral Metabolism
2. Section on Developmental Neurochemistry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regulation of protein synthesis in the brain

Previous Serial Number: Same

Principal Investigator: Michael J. Schmidt, Ph.D.

Other Investigators: Louis Sokoloff, M.D., Mr. J. D. Brown

Cooperating Units: None

Man Years:

Total:	2.25
Professional:	0.75
Other:	1.50

Project Description:

Objectives: One of the most prominent biochemical changes occurring in the brain during maturation and development is a change in the rate of protein synthesis. Earlier work in this and other laboratories has demonstrated in the rat that cerebral protein synthesis proceeds rapidly in the early postnatal period but declines markedly as maturation is achieved. The initial objective of this project is the elucidation of the control mechanisms responsible for this fundamental biochemical change with maturation.

Methods Employed: Protein synthesis is measured in cell-free preparations from brains of rats of various ages from birth to maturity. The soluble enzymes and the ribosomes, which comprise the cellular biochemical machinery for protein biosynthesis, are separated and purified by a variety of

ultracentrifugation techniques and by isolectric precipitation methods. These are recombined in reaction mixtures containing the necessary energy sources, cofactors, and radioactive amino acids to permit the process of protein synthesis to proceed under optimal or test conditions. The direct effects of cyclic AMP and cyclic GMP on brain protein synthesis are examined under optimal conditions which have been established in previous experiments. The influence of a cyclic AMP-dependent enzyme, protein kinase, on the process of protein synthesis is also under investigation. The protein kinase enzyme is purified from rat brain by isoelectric precipitation, salt fractionation and column chromatography. Ribosomes isolated from the brains of rats at various ages are characterized by sucrose gradient ultracentrifugation and UV absorbancy. Purified ribosomes are incubated in the presence of cyclic AMP, purified cyclic AMP-dependent protein kinase, and ^{32}P-ATP, and the ability of these ribosomes to be phosphorylated is determined. Both endogenous protein kinase activity and phosphate acceptor activity of the various cellular fractions involved in protein biosynthesis are examined as a function of age. Particular attention is being given to the ribosomes since these cellular components are suspected to change functionally during the development of the brain. With the optimal purification procedures and assay conditions for cyclic AMP-stimulated ribosomal phosphorylation and for protein synthesis established, it has become possible to examine under tightly controlled conditions the effects of phosphorylation or ribosomal protein on brain protein biosynthesis.

Major Findings: Previous work in this laboratory demonstrated that protein synthesis *in vitro* in cell-free preparations from rat brain declined about 75% between the ages of 15 days and 45-60 days. During this interval the major part of postnatal development and maturation of the brain occurs. These *in vitro* data are in close agreement with the results obtained by others *in vivo*, but the *in vitro* methods permit more definitive studies of the mechanism of the change. Other laboratories since have demonstrated that there is a progressive decline in protein synthesis in the brain with maturation and have implicated functional changes in the ribosomes as being, at least partly if not completely, responsible for the change.

The principal investigator has previously found an almost inverse pattern of change of the cyclic AMP system in developing rat brain. This system is relatively inactive at birth and then rises to the adult level during maturation of the brain. Cyclic AMP is known to stimulate the activity of a protein kinase, an enzyme(s) which catalyses the phosphorylation of many proteins. The question has, therefore, been raised whether the cyclic AMP-stimulated protein kinase may cause phosphorylation of proteins of the protein synthesizing system and thus modify their activity in protein synthesis. In particular, the hypothesis being tested is that the development of the cyclic AMP system during maturation of the brain leads to increased phosphorylation of ribosomal structural or functional proteins, thus causing lower ribosomal activity in protein synthesis with maturation.

The results of this project have demonstrated that microsomes of brain contain protein kinase activity which is stimulated by cyclic AMP, but no differences were observed with age of the animal. The development of this enzyme in brain was not altered by early administration of thyroid hormones which are known to accelerate the development of a number of other neurophysiological and biochemical events in the brain.

With a more refined assay system it has been found that ribosomal proteins are fully capable of being phosphorylated by a brain protein kinase, and this phosphorylation is dramatically stimulated by cyclic AMP. These studies have also demonstrated that ribosomes at all ages can be phosphorylated to the same degree; no differences in phosphate acceptor ability of ribosomes were noted from birth to 1 year of age.

A protein synthesizing system has been developed which permits control over the various components in the protein biosynthetic process. In this optimalized system addition of cyclic AMP and cyclic GMP produced a moderate stimulation of protein synthesis, but only at elevated concentrations. In this same system ribosomes phosphorylated by the cAMP-stimulated protein kinase have been found to have essentially unchanged protein synthetic activity. It has been observed, however, that the phosphorylated ribosomes become dephosphorylated by a phosphatase during the incubation for protein synthesis. This complication must be resolved before a definitive answer to the initial question is achieved.

Significance to Bio-medical Research and the Program of the Institute: Protein synthesis is a key process involved in the development and maturation of all tissues, including the brain, as well as in the renewal of their intracellular components and in regulation of their biochemical processes in response to physiological, pharmacological, and pathological stresses. A number of syndromes of mental retardation are clearly the result of some disturbance in the process of protein synthesis or its regulation in the brain during its maturation. It is hoped that these studies will shed some light on the normal mechanisms of regulation of protein synthesis in the brain during maturation so that more directed and relevant studies of the biochemical bases of the abnormalities in mental retardation can be undertaken. Furthermore, a good deal of current evidence suggests that cyclic AMP and cyclic GMP might be the intracellular mediators of the putative neurotransmitters, norepinephrine and acetylcholine, respectively. Alterations in the concentrations of both of these transmitters in certain affective disorders and following the administration of psychoactive drugs have been documented. At the present time it is unknown how the neurotransmitters exert their effects on brain function. Effects at the level of protein synthesis could have wide ranging consequences on many aspects of neuronal functioning. The present experiments are designed to determine whether such alterations in protein biosynthesis can in fact be brought about as a consequence of alteration in the concentration of cyclic AMP or cyclic GMP.

Proposed Course of Project: Studies will be continued to determine if cyclic AMP-stimulated phosphorylation of ribosomal proteins alters the ability of the ribosomes to function in protein synthesis, and to determine if such an effect is age dependent. As soon as these questions are answered, the project will be temporarily suspended because of lack of professional personnel.

Honors and Awards: None

Publications:

Schmidt, M. J., and Sokoloff, L.: Activity of cyclic AMP-dependent microsomal protein kinase and phosphorylation of ribosomal protein in rat brain during postnatal development. J. Neurochem. 21: 1193-1205, 1973.

Schmidt, M. J.: Effects of neonatal hyperthyroidism on activity of cyclic AMP-dependent microsomal protein kinase. J. Neurochem., 1973 (in press).

Serial No. M-CM-MyC-1
1. Laboratory of Cerebral Metabolism
2. Section on Myelin Chemistry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical studies on myelin and myelin basic protein

Previous Serial Number: Same

Principal Investigator: Russell E. Martenson

Other Investigators: Marian W. Kies and Gladys E. Deibler

Cooperating Units: Department of Pathology, New York Medical College Center for Chronic Disease, Bird S. Coler Hospital, Welfare Island, New York.

Man Years:

Total:	3.5
Professional:	1.0
Others:	2.5

Project Description:

Objectives: To examine the relationships between chemical structure and biological activities of the myelin basic proteins of several mammalian and submammalian species. The biological activities include the ability to (A) induce delayed hypersensitivity leading to an autoimmune disease, experimental allergic encephalomyelitis (EAE), (B) induce delayed hypersensitivity without necessarily causing EAE, (C) induce the formation of circulating anti-basic protein antibodies without concomitant induction of EAE, and (D) provide structural stability in myelin by its interaction with lipids in the membrane.

Methods Employed: (1) Polyacrylamide gel electrophoresis; (2) amino acid analysis of CNS proteins and their derivatives; (3) chromatographic fractionation of proteins and peptides; (4) paper chromatography; (5) high voltage electrophoresis; (6) thin-layer chromatography; and (6) specific chemical and enzymic cleavage of myelin basic proteins.

Major Findings: (A) A major part of the research has been the preparation of specific fragments of myelin basic proteins for use in chemical and immunological studies. A great deal of effort has been spent in their isolation in highly pure form and in their characterization. In collaboration with Dr. Seymour Levine, Bird S. Coler Hospital, New York, we have been using these fragments to locate the regions of the guinea pig and bovine protein which induce EAE in Lewis rats. These studies are of importance because they

provide a general picture of what constitutes an encephalitogenic determinant. A determinant which is capable of inducing a state of cell mediated immunity leading to an autoimmune disease depends upon the species of animal used in the bioassay. The principal, if not the sole, determinant for EAE in the guinea pig has been identified as a nonapeptide containing the single tryptophanyl residue of the protein. All mammalian basic proteins of molecular weight 18,000-19,000 contain this region and all are equally active in guinea pigs. The homologous proteins of chicken, turtle, and frog origin, which have major modifications of this region are virtually inactive at all dose levels. In contrast, Lewis rats do not respond to this region of the basic protein at all. Furthermore, they show a differential sensitivity to basic proteins of different species. Specifically, basic proteins of guinea pig and rat origin are extremely active, whereas basic proteins of human, bovine, rabbit, monkey, chicken, turtle, and frog are active only at much higher dose levels. This can be interpreted to mean either that (1) Lewis rats, as distinct from guinea pigs, recognize a single site whose phylogenetic modification markedly lowers the activity but does not eliminate it or that (2) Lewis rats recognize two entirely different sites. The extremely active site would be present only in the rodent proteins, whereas the weaker one would be present in the protein of many species.

Our studies involving fragments obtained from the basic protein by specific cleavages with pepsin have shown that the extremely high activity of the guinea pig protein in Lewis rats is associated solely with the fragment constituting residues 44-89. The corresponding fragment obtained from the bovine protein is inactive. The all or none species difference in activity of this fragment excludes possibility (1) above and shows that possibility (2) is correct. The activity of the bovine protein can be accounted for by the chemically-derived fragment comprising residues 21-116. In addition, significant activity is associated with peptic fragments 1-89, 90-170, and 90-153. No significant activity is associated with peptic fragments 1-43, 44-89, and 112-170, or with the chemically-derived fragment 117-170. These results indicate that there are at least two weak encephalitogenic determinants in the bovine protein. One site must begin after residue 20 and extend beyond residue 44, while the other must begin after residue 89 and terminate before residue 154.

The presence of two mutually exclusive encephalitogenic sites in the bovine protein of roughly equal activity suggests that the two regions may be similar in their amino acid sequences. Alignment of residues 35-47, a sequence which must include at least part of the major encephalitogenic site, with residues 108-119, which are contained within the second active region, shows a striking sequence similarity. Aside from 3 deletions, there are 7 identities and 4 conservative interchanges, 3 of which require only a single nucleotide base change in the genetic code:

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 35                                                  47
-Gly-Ile-Leu-Asp-Ser-Leu-Gly-Arg-Phe-----Phe-Gly-Ser-Asp-
 108                                                 119
-Gly-----Leu-----Ser-Leu-Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-
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(B) The second major endeavor in our laboratory has been to explain the microheterogeneity of myelin basic protein. Studies by Mrs. Deibler have shown that 3 major forms of basic protein exist by virtue of missing arginyl residues at the C-terminus of the protein. The completely intact polypeptide chain, Component 1, has the C-terminus -Ala-Arg-Arg; Component 2, -Ala-Arg; and Component 3, -Ala. The completely intact polypeptide chain (C-terminus, -Ala-Arg-Arg) also exists in multiple forms by virtue of phosphorylated serine and/or threonine residues. Component 1 is phosphate-free, Component 3 contains 1 phosphorylated residue, and Component 5, 2 phosphorylated residues. Component 4 appears to be a form with C-terminus Ala-Arg containing 1 phosphorylated residue. Thus the microheterogeneity arises from a combination of two factors: loss of C-terminal arginyl residues and phosphorylation.

Significance to Biomedical Research and the Program of the Institute: Myelin basic protein is under intensive investigation in this and a number of other laboratories to determine its role in the structural integrity of the myelin sheath, where it accounts for 30% of the myelin proteins. Closely related to this role is its involvement in an experimental autoimmune disease of the CNS, EAE, where it serves as a potent auto-antigen capable of specifically sensitizing lymphocytes which initiate a delayed-type hypersensitivity reaction in the CNS tissues leading to paralytic lesions. To study the properties of the protein responsible for the induction of the delayed hypersensitivity, both with and without concomitant production of EAE, and those responsible for induction of antibody formation alone, we have examined various areas of the basic protein for the relevant activities. Through these studies we hope to obtain information relevant to molecular events which occur in the afferent part of immune responses in general, and possibly during the early phases of autoimmunity in human, specifically in multiple sclerosis.

Proposed Course of Project: (A) Immunological studies: Further studies will be carried out to define more specifically the determinants of the guinea pig and bovine proteins which are encephalitogenic in Lewis rats. The fragments of the basic proteins which have so far enabled us to localize the encephalitogenic determinants to certain, rather large, regions of the basic proteins will be further cleaved by specific chemical and enzymatic means. The resulting fragments, in addition, will be of immense value in locating those regions which are involved in the induction of circulating antibodies in guinea pigs. These latter studies are being carried out by Dr. Driscoll.

(B) Chemical studies: The additional fragments obtained from the basic protein will be used to locate the positions in the protein where enzymatic phosphorylation of amino acids takes place, both *in vivo* and *in vitro*. These studies should provide us with information relating to the metabolism and function of this protein in the myelin sheath.

Honors and Awards: None

Publications:

Martenson, R. E., Levine, S., Deibler, G. E., and Kramer, A. J.: Comparative studies of guinea pig and bovine myelin basic proteins. Partial characterization of chemically derived fragments and their encephalitogenic activities in Lewis rats. J. Neurochem., in press.

Serial No. M-CM-MyC-2
1. Laboratory of Cerebral Metabolism
2. Section on Myelin Chemistry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunological studies on experimental allergic encephalomyelitis (EAE)

Previous Serial Number: Same

Principal Investigator: Marian W. Kies

Other Investigators: Martha L. Bacon, E. C. Alvord, Jr., F. J. Seil, Henry D. Webster

Cooperating Units: Dept. of Pathology, University of Washington, Seattle; Dept. of Neurology, VA Hospital, Palo Alto, Calif.; Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS

Man Years

Total:	2.5
Professional:	1.0
Others:	1.5

Project Description:

Objectives: To study specific serum factors in autoimmune phenomena in rabbits.

Methods Employed: Induction of precipitating and/or nonprecipitating antibody to myelin basic protein (BP) in rabbits by manipulation of dose of antigen, timing of injections, use of Freund's adjuvant with or without mycobacteria, etc.

These sera are being used for various exploratory studies pertaining to (A) structural requirements for antibody induction in rabbits; (B) relation of humoral antibody to disease induction; (C) presence and identity of serum factors responsible for damage to CNS tissue in vitro; or (D) damage to Xenopus tadpole optic nerve in vivo.

Major Findings: (A) Specificity of anti-myelin basic protein antibody induced in rabbits: This study of antibody production in rabbits was begun originally to prepare specific antibody for chemical investigations of BP, e.g., detect amino acid sequence changes from species to species, detect traces of impurities, otherwise undetectable, assess the purity of peptide fragments and relate antibody-binding sequences to encephalitogenic sequences.

Added benefits of the large collection of sera prepared by various immunization schedules are discussed in (B), (C), and (D) below.

The following antigens have been used for antibody induction: several different myelin basic proteins (mammalian and submammalian); peptide fragments derived from guinea pig basic protein (GPBP); whole CNS tissue (bovine and frog spinal cord); pure cerebroside; and complete Freund's adjuvant (CFA) which contains killed mycobacteria.

Mammalian BPs all induce antibodies which cross-react completely with all other mammalian BPs tested. Limited cross reactions have been detected between mammalian and submammalian proteins; chicken BP induces sera which react well with mammalian BPs but chicken BP reacts poorly with antisera induced by mammalian BPs. There is also limited cross reactivity among the few submammalian BPs we have tested. Antisera to mammalian BPs show variable cross reactions with fragments of guinea pig BP--i.e., some antisera precipitate fragments 1-89 and 44-89, but not fragment 90-170. Other antisera precipitate both fragments 90-170 and 1-89, but not fragment 44-89, etc.

Whole tissue immunization induces anti-cerebroside antibody but no anti-BP; cerebroside immunization has not yet induced detectable antibody to cerebroside; CFA alone does not induce antibodies which react with BP.

(B) Relation between humoral antibody to BP and onset of EAE: We have now accumulated adequate information to conclude there is no correlation between onset of EAE and levels of circulating (precipitating) antibody. In the group of over 70 rabbits, some animals had prolonged periods of antibody production without evidence of EAE, others failed to produce antibody, even after repeated immunizations, and also showed no signs of EAE, others produced no antibody and succumbed to EAE, or produced high levels of antibody at the time of disease onset. Most sera have been analyzed only for precipitating antibody. At the present time we are analyzing negative sera for non-precipitating antibody which can be detected by radioimmunoassay.

(C) Identification of serum factors concerned with demyelination in vitro (with Dr. Seil): Our previous studies in collaboration with Dr. Seil showed that basic protein immunization of guinea pigs does not induce antisera containing anti-myelin factor whereas antisera induced by whole cord immunization prevent myelination and cause demyelination in vitro (i.e., contain anti-myelin factor). We concluded, therefore, that the anti-myelin factor induced by whole cord was not related to EAE.

We have extended this study to rabbit sera of known antibody content. In a series selected for various combinations of antibody and disease, Dr. Seil found anti-myelin factor in 7 of 29 coded sera we supplied him. The results are summarized in the Table that follows. Other investigators who have repeatedly reported anti-myelin factor in "EAE sera" have recently reported that anti-cerebroside antibody is the anti-myelin factor. The data in the Table suggest that other factors may also be involved.

Anti-myelin activity	Antigen	Clinical EAE	Anti-BP
Strongly positive (5 sera)	Whole bovine cord (3 sera)	+	-
	Rabbit BP[1]	-	+
	Rat-S BP[2]	+	+
Intermediate (2 sera)	Human BP	-	+
	Fragment 44-89[3]	+	-
Negative (19 sera)	Rabbit BP[1]	-	-
	Rat-S BP[2]	+	+
	Fragment 44-89[3]	+	-
	Normal sera (4 sera)	-	-
	CFA controls (2 sera)	-	-
	GPBP (2 sera)	-	+
	Bovine BP (2 sera)	-	+
	Monkey BP (2 sera)	+	-
	Monkey BP	-	+
	Human BP (2 sera)	-	-

[1,2,3] Denote sera from pairs of rabbits receiving identical injections.

(D) Identification of serum and cell factors concerned with demyelination in vivo (Dr. Webster): This study is still in a preliminary stage. Dr. Webster has developed a simple whole mount technique for examining the optic nerve of Xenopus tadpoles. The technique is superior to the in vitro tissue culture methods because of the controlled internal milieu, the simplicity of handling tadpoles (relative to tissue culture) and the ability to assess damage quantitatively. It has been used in Dr. Webster's laboratory to study hexachlorophene poisoning, crush injury, etc.

We are providing lymph node cells from sensitized guinea pigs for tests of their ability to induce lesions in this species. If it is possible to demonstrate a clear relationship between sensitization (either cellular or humoral) and lesion formation it will be possible to analyze and possibly identify the anti-myelin factor which has been shown to occur in multiple sclerosis (MS) patient's serum.

Significance to Biomedical Research and the Program of the Institute: Highly specific precipitating antibody is a powerful tool in the investigation of details of protein structure as well as for the detection of traces of contaminating proteins in purified protein preparations.

During the past year, the President's Commission on Multiple Sclerosis has recommended increased support of research in demyelinating diseases, particularly with respect to an effective treatment for MS. Studies are underway in various laboratories, including our own, laying the groundwork for clinical trials of myelin basic protein as a means of blocking putative autoimmune reactivity in MS patients. Our studies on antibody specificity are

extremely important in the development of an objective procedure for monitoring such trials (in addition to subjective evaluation of patient reaction to myelin basic protein treatment).

Proposed Course of Project: The availability of specific antisera has facilitated our collaboration in two very important studies of the relevance of anti-myelin factors to demyelination (C and D above). Although these factors are considered to be important in human demyelinative diseases, no rational proof of their role in pathogenesis has been provided. We have been limited in our investigation of this problem in the past but we feel that the current collaborative studies with Drs. Seil and Webster will permit more definitive studies.

Honors and Awards:

Dr. Kies received a Travel Award from The Kroc Foundation to attend the Fourth International Meeting of the International Society for Neurochemistry, Tokyo, Japan, 1973.

Publications:

Behan, P. O., Kies, M. W., Lisak, R. P., Sheremata, W., and Lamarche, J. B.: Immunologic mechanisms in experimental encephalomyelitis in nonhuman primates. Arch. Neurol. 29: 4-9, 1973.

Kies, M. W.: Experimental allergic encephalomyelitis. In Gaull, G. E. (ed.): Biology of Brain Dysfunction. New York, Plenum Press, 1973, Vol. 2, pp. 185-224.

Serial No. M-CM-MyC-3
1. Laboratory of Cerebral Metabolism
2. Section on Myelin Chemistry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on immediate and delayed hypersensitivity in experimental allergic encephalomyelitis (EAE)

Previous Serial Number: Same

Principal Investigator: Bernard F. Driscoll (Guest Worker)

Other Investigators: Marian W. Kies and Allan J. Kramer (Guest Worker)

Cooperating Units: National Multiple Sclerosis Society

Man Years

Total:	3
Professional:	1
Others:	2

Project Description:

Objectives: To study the relationship of protein structure to immunogenicity--cellular versus humoral response to myelin basic protein (BP) in the development of the autoimmune response (EAE).

Methods Employed: (1) Induction of EAE has been used as a tool to study mechanisms of prevention or treatment of autoimmune disease. These mechanisms have been further elucidated at the cellular level by combining treatment with passive transfer. (2) Highly purified peptide fragments of BP combined with radioimmunoassay have been utilized to locate sites on the protein molecule responsible for its immunological activity.

Major Findings: (A) We have reported the effectiveness of treatment with BP after EAE onset in guinea pigs. The treatment was successful in isogenic Strain 13 guinea pigs at both high and intermediate levels of sensitization and in random bred NIH guinea pigs at an intermediate level of sensitization. The disappearance of clinical signs of EAE was rapid--usually within 24-72 hours after beginning the series of BP injections. There was no obvious explanation for the mechanism involved.

The high level of sensitization (0.5 mg BP/2.5 mg MB) used in the treatment study was the same as that used to sensitize donors for the production of lymph node cells in passive transfer experiments. In a study combining treatment of donor guinea pigs with passive transfer of sensitized lymph node cells to naive recipients, we have shown that at least one mechanism

involved in treatment involves its effect on sensitized lymphocytes. Within 48 hours after the start of donor treatment their lymph node cells begin to lose their ability to transfer EAE. By 72 hours after the start of treatment donor cells are no longer capable of causing EAE in recipients. At this time there is also obvious improvement in the clinical state of the donor guinea pigs. It is clear, therefore, that rapid reversal of disease signs is related to the fact that sensitized cells are no longer being released into the circulation from lymph node depots. The site of action of the basic protein treatment involves lymphocytes rather than the CNS.

(B) Experiments involving passive transfer have also been used to analyze the immunologic reactions involved in protection. If an animal is repeatedly injected with basic protein in incomplete Freund's adjuvant (IFA) before sensitization with the protein in complete Freund's adjuvant (CFA), the expected disease state does not develop. We have shown that this protection against EAE induction also prevents passive transfer of EAE by a highly encephalitogenic injection of lymph node cells. Protection against active sensitization lasts longer than protection against passive sensitization. EAE cannot be induced in protected animals by active immunization for periods of ~6 months after priming whereas protection against passive transfer of cells is lost 5-6 weeks after priming.

(C) We have continued to investigate the structure of regions of the BP molecule responsible for its immunological reactivity. These include sites that bind antibody, sites that induce antibody and sites that induce cell mediated immunity and/or EAE. The sites which bind antibody have been located in the regions between residues 44 and 89, residues 90 and 116, and residues 117 and 156. The demonstration of mutually exclusive antigenic regions on the molecule capable of binding antibody provides a clue to the types of cells involved in antibody production (B-cells) but yields no information about the specificity of antibody induction or development of delayed hypersensitivity, both thought to be thymus-dependent (T-cell) functions. Whether they are joint or alternate functions of a single clone of cells or functions of two different clones of cells is not clear.

It has been recognized that production of antibody involves two separate mechanisms, the preparative phase or priming and the inductive phase or elicitation of antibody. The specificities of these two have been shown to involve different sites in studies with synthetic antigens. In an analysis of comparable mechanisms in EAE, we have used the terms, <u>priming</u>, with reference to injections of BP in IFA and <u>induction</u> with reference to the challenge with BP in CFA. The latter injection in normal guinea pigs induces EAE accompanied by delayed hypersensitivity but induces very little antibody. When preceded by the <u>priming</u> injections, an injection of BP in CFA induces antibody, but does not induce EAE or delayed hypersensitivity to BP.

Priming controls the specificity of the antibody produced (see Table). When peptides derived from BP are used for priming and whole BP is used for induction, the antibodies bind primarily only those sites contained in the

peptide used for priming; when whole BP (1-170) is used for both priming and induction, the antibodies bind all regions of the molecule (L, C, R).

Prime With	Antibodies Specific To		
	L	C	R
1-90 (L)	+	-	-
90-170 (C,R)	-	+	+
117-170 (R)	-	-	+
1-170 (L,C,R)	+	+	+

The location of the site(s?) on the molecule responsible for antibody induction has been similarly investigated. Guinea pig basic protein contains the site required for induction whereas rat S basic protein (which lacks the peptide sequence corresponding to residues 117-156 of the guinea pig protein) cannot induce antibody and therefore lacks all or part of the inducing site. However, at least two of the specific antigenic regions found in guinea pig basic protein are present intact in the rat S molecule; therefore, priming with rat S results in antibody possessing specificity for these two regions (L and C).

Significance to Biomedical Research and the Program of the Institute: Knowledge of the mechanisms involved in the induction, prevention and cessation of immunologic reactions to myelin basic protein as well as the interrelationships between cellular and humoral immunity are a prerequisite to an understanding of autoimmune diseases, in general, and, in particular, are important for developing a rational plan for immunologic intervention in multiple sclerosis. Even if the precipitating event in this disease does not involve an autoimmune reaction to BP, the repetitive course of the illness suggests an autoimmune reaction to some antigen, and the techniques developed would be of use with any antigen.

Proposed Course of Project: We plan to continue our analysis of the cell types responsible for disease induction and to investigate techniques for controlling their proliferation once the antigenic stimulus is established. Which cell types (T or B) in the sensitized lymph node population are responsible for EAE induction in normal recipients? What is the nature of the cells or cell factors that suppress the disease state? More work is planned to answer these questions and to study the relationship of structure to protection of guinea pigs against either passive or active induction of EAE.

Honors and Awards: None

Publications:

Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr.: Successful treatment of experimental allergic encephalomyelitis (EAE) in guinea pigs with homologous myelin basic protein. J. Immunol. 112: 392-397, 1974.

Driscoll, B. F., Kramer, A. J., and Kies, M. W.: Myelin basic protein: location of multiple independent antigenic regions. Science 184: 73-75, 1974.

Serial No. M-CM-MeC-1
1. Laboratory of Cerebral Metabolism
2. Section on Membrane Chemistry
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974 .

Project Title: Study of the modulation of central nervous system metabolism and function through alterations in membrane permeability and transport

Previous Serial Number: Same

Principal Investigator: William C. Torch, M.S., M.D.

Other Investigators: James D. Brown, Carolyn S. Cottingham, and Louis Sokoloff, M.D.

Cooperating Units: None

Man Years:

Total:	2.75
Professional:	1.25
Other:	1.5

Project Description:

Objectives: Neurotransmitters exert their effects in the central nervous system (CNS) by altering the state of CNS post-synaptic membranes. This might occur through transient and/or permanent alterations in the lipid and protein components of the membrane via metabolic mechanisms as well as through intra-membranar lipid-protein phase changes, either of which might affect ion permeability and electrical excitability. Previous workers in this laboratory have already demonstrated that the neurotransmitter, acetylcholine (ACh), can affect phospholipid synthesis and degradation in CNS membranes (synaptosomes). The molecular mechanism by which ACh causes these effects has been the intense objective of study of this laboratory. Specifically, acetylcholine has been demonstrated to increase the rate of

incorporation of radiophosphate into phosphatidylinositol (PI) and phosphatidic acid (PA). Addition of ACh has been shown to enhance the hydrolysis of PI and its derivatives diphosphophosphatidylinositol (DPI) and triphosphophosphatidylinositol (TPI) into their inositol phosphate breakdown products. Calcium has been shown to affect the rate at which ACh-mediated phospholipid synthesis and degradation occur. The mechanism by which ACh alters phospholipid synthesis has still further to be elucidated.

It has been noted in the literature that norepinephrine, as well as other putative neurotransmitters, has the same effects as ACh on phospholipid metabolism, i.e., the "phospholipid effect" is not specific to ACh. Moreover it has been noted over the past few years that some cyclic nucleotides might be specific mediators of the neurotransmitters in the post-synaptic intramembranar cellular elements. Cholinergic mechanisms may be specifically mediated through the cyclic GMP-guanylylcyclase enzyme system, and adrenergic mechanisms through the cyclic AMP-adenylylcyclase enzyme system. The question that logically follows, and to which this project is addressed, is whether acetylcholine and cholinergic drugs affect phospholipid synthesis through the cyclic GMP system, and whether norepinephrine and other adrenergic drugs express their effects on phospholipid metabolism through the cyclic AMP system.

Methods Employed: The project is to be approached in 3 stages, the first two of which are already in progress.

1. To determine if cyclic GMP and cyclic AMP and their derivatives can in themselves affect phospholipid metabolism in synaptosomes. Also to determine whether cyclic GMP and cyclic AMP can affect the cholinergic and adrenergic mediated phospholipid response induced by acetylcholine and norepinephrine, respectively.

2. To determine whether ACh and norepinephrine and other putative neurotransmitters (epinephrine, serotonin, histamine, dopamine, GABA) do indeed affect levels of the cyclic nucleotides in synaptosomes. In order to do this, methods for the separation, purification and measurement of cyclic AMP and cyclic GMP will be established.

3. To determine whether the phospholipid response mediated by ACh and the other neurotransmitters is limited to specific areas in the brain that are specialized in specific cyclic nucleotide synthesizing ability.

In Stage 1, synaptosomes isolated from guinea pig cortex by differential centrifugation are incubated for varying time periods in the appropriate radiophosphate-(^{33}P) media containing acetylcholine, cyclic AMP and cyclic GMP in various concentrations. By established lipid-extraction techniques the various phospholipids are isolated and purified by thin-layer chromatography and then measured for radioisotope incorporation. A measure of how much effect each of the aforementioned agents has in phospholipid metabolism is obtained.

In Stage 2 synaptosomes isolated from guinea pig cortex will be incubated with each of the putative neurotransmitters. Cyclic nucleotides will be separated and purified by column chromatography. Cyclic AMP will be assayed by using a radioisotope displacement technique employing a purified preparation of cyclic AMP dependent-protein kinase. Cyclic GMP production will be assayed by measuring the rate of formation of [^{3}H]-cGMP from its precursor [^{3}H]-GTP and by measuring the phosphorylation of histone mediated by a purified preparation of cyclic GMP-dependent-protein kinase.

In Stage 3 various areas of brain will be assayed for the effects of ACh and other neurotransmitters on cyclic nucleotide production (as in Methods, Stage 2). Also the effect of cyclic nucleotides on phospholipid metabolism in synaptosomes isolated from these brain regions will be measured (as in Methods, Stage 1).

Major Findings: Our working hypothesis is that ACh exerts its effects on phospholipid turnover secondarily to its stimulation of the production of cyclic GMP by direct activation of intramembranar guanylylcyclase. Cyclic GMP might then interact at some level of phosphatidylinositol metabolism causing a markedly increased turnover of phosphatidylinositol and its precursor, phosphatidic acid. Likewise norepinephrine and histamine might enhance cyclic AMP production through similar mechanisms involving adenylylcyclases. Cyclic AMP might then have similar phospholipid effects.

Last year it was noted in our preliminary findings that cyclic GMP (cGMP) and acetylcholine (ACh) both stimulated ^{33}P-radiophosphate into synaptosomal phospholipids from guinea pig cortex synaptosomes. The effects of cGMP were dose-dependent; high concentrations were inhibitory, and low concentrations stimulatory. The findings, however, were complicated by the fact that theophylline, a phosphodiesterase inhibitor added to the incubation media to prevent hydrolysis of cGMP during the incubation, had complicating effects of its own. Rather than pursue a concurrent study on the effects of theophylline, phosphodiesterase-insensitive dibutyryl derivatives of cGMP and cAMP were used in lieu of the combination of cGMP plus theophylline.

Synaptosomes were isolated and incubated in the media of Schacht and Agranoff (J. Biol. Chem. 247:771, 1972). Effects of addition of dibutyryl-cGMP (dbcG), -cAMP (dbcA) (10^{-7}-10^{-2}M), or ACh on phospholipid turnover were determined by measuring $^{32}P_i$ incorporation into PI, phosphatidyl-choline, -serine, -ethanolamine and phosphatidic acid (PA). DbcG, dbcA, and ACh stimulated $^{32}P_i$ into PI. Maximum stimulation was at 5×10^{-6}M dbcG, 10^{-4}M dbcA and 10^{-4}M ACh; effects were seen down to concentrations of 10^{-7}M dbcG. Whereas dbcG also stimulated PA turnover, dbcA had no such effect.

These data indicate that cGMP and cGMP- and cAMP-related derivatives do affect PI turnover. Whether ACh produces its phospholipid effects by increasing levels of cGMP in our synaptosome preparation is under study. If cGMP levels were to rise as a result of ACh stimulation, it could conceivably occur by either of 2 mechanisms: stimulation of guanylylcyclase activity or inhibition of phosphodiesterase activity, or both. In most tissues where ACh effects cGMP levels, guanylylcyclase activity is stimulated. For this reason the guanylylcyclase activity of synaptosomes is under study.

Synaptosomes are incubated in the presence and absence of ACh. The rate of formation of [^{3}H]-cGMP from its precursor [^{3}H]-GTP will be radioactively assayed, after chromatographic isolation of cGMP by using the methods of Bensinger and Chader (J. Neurochem., in press). The measurement of cGMP levels by the protein kinase assay of Mao and Guidotti (Analyt. Biochem., in press) and Kuo and Greengard (J. Biol. Chem. 245:2493, 1970) has

been abandoned because of difficulties in measuring the very low levels of cGMP in our synaptosome preparation, and because of the important reason of measuring guanylylcyclase rather than net cGMP production as pointed out above. The ability of the cortex to respond to both dibutyryl cyclic GMP and to a much lesser degree to dibutyryl cyclic AMP in a similar non-specific manner may be a reflection of the presence of both cholinergic and adrenergic receptors in this area of the brain.

Significance to Bio-medical Research and the Program of the Institute: The mechanism of action of neurotransmitters in the CNS and other target organs is presently unknown. In addition to the theoretical interest of how transmitters affect electrical activity in the nervous system, knowledge of the mechanism of hormone action would shed light on the physiological and pathological mechanisms of health and disease in the nervous system. Recently it has become apparent that altered levels of cyclic nucleotides are observed in altered psychologic states, and that psychotropic drugs can alter levels of cyclic nucleotides. Knowledge of the mechanism would help to elucidate how pharmacologically active drugs work, and would provide a rational basis for the development of new drugs to be used in treating disease.

Proposed Course of Project: Further studies on the mechanism of action of neurotransmitters and their biochemical intermediates on phosphoinositide and phospholipid metabolism in the CNS are to be continued as described in Methods.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 18
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the Structure and Function of Thetin-Homocysteine Methylpherase and Lactose Synthetase

Previous Serial Number: Same

Principal Investigator: Werner A. Klee

Other Investigators: K. Subramonia Iyer and Claude B. Klee

Cooperating Units: Laboratory of Biochemical Pharmacology, NIAMDD

Man Years:

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives: Macromolecular complexes on many levels are a central principle of biological organization. This project is aimed at learning how the properties of such complexes can differ from those of the isolated components in two enzyme systems. One enzyme, thetin-homocysteine methylpherase, exhibits a number of interactions of like protein species; the other, lactose synthetase, is an example of an enzyme in which two very different protein species interact to form the whole.

Methods Employed: We are using the standard method of protein chemistry including chromatography, electrophoresis, preparative and analytical ultracentrifugation, spectrophotometry and spectropolarimetry. In addition, many of the standard analytical techniques of enzymology are being employed.

Major Findings: Lactose synthetase consists of two protein components both of which are found in lactating mammary glands and in milk. The two proteins are the A-protein and α-lactalbumin. We have isolated the A-protein from milk and shown it to be a mixture of closely related glycoproteins.

The A-protein alone can carry out galactose transfer reactions from UDP-galactose to a variety of glycoproteins, glycolipids and to some simple sugars such as N-acetyl-glucosamine. The effect of α-lactalbumin on the system is to produce a dramatic increase in the affinity for sugar acceptors be they glucose or N-acetylglucosamine. This simple picture is complicated by the existence of a substrate inhibition seen at high concentrations of acceptor sugars. α-Lactalbumin enhances this effect as well and so is seen to stimulate or inhibit glycosyl bond formation depending on the acceptor sugar concentration.

These kinetic observations made it appear highly likely that α-lactalbumin functions by combining with the A-protein to form a multisubunit complex. We have found that such complexes are indeed formed but only in the presence of one of the substrates of the enzyme, either UDP-galactose or N-acetylglucosamine. Complex formation was demonstrated by a number of methods including a newly developed modification of band sedimentation in the analytical ultracentrifuge. The complex contains one molecule of A-protein and one molecule of α-lactalbumin and is quite easily dissociated. Although α-lactalbumin is the only protein known to function in this system, it has been found that the only modifications to its structure which will prevent interaction with the A-protein are major ones such as disulfide bond reduction or extensive proteolysis. Modification of amino acid residues such as tyrosine, methionine, histidine and at least some aspartic and glutamic acids results in only a minor decrease in activity. Thus, the interaction of the two proteins may involve the mutual recognition of large areas of their surfaces. Studies of the forces involved in stabilizing the complex show that ionic interactions may play a major role, and that hydrophobic bonding is probably not an important contributor to the binding energy.

During the course of a study of the guanidination of α-lactalbumin by o-methylisourea, a number of important properties of the protein have been uncovered. We can now say that all 12 lysine residues of the protein can be converted to homoarginine without sensibly changing its overall structure or function. Thus, lysine residues, as such, are not an essential part of the interaction of α-lactalbumin and the A-protein. A side reaction during guanidination is disulfide interchange leading to polymer formation and loss of function. α-Lactalbumin is unique in this respect and it was therefore plain that its disulfide bonds exhibit an unusual degree of reactivity. Measurement of the rate of

reduction of the four disulfide bridges of α-lactalbumin, with the aid of a direct spectrophotometric assay developed by us for this purpose has shown that the disulfides of this protein are indeed extraordinarily reactive. In particular, one disulfide bridge, linking cysteinyl residues 6 and 119 at the amino acid carboxyl termini of the molecule is highly reactive even at 0^{o}. The other three disulfides are reactive at room temperature and above but not at low temperatures. These results bring into question the similarity of structure of α-lactalbumin and lysozyme which has been postulated on the grounds of amino acid sequence homology.

An activity similar to or identical with that of the A-protein found in milk has been reported to be present in particulate fractions of many tissues. We have therefore initiated an examination of the properties of this enzyme in a number of tissues but have concentrated our attention on brain. In the rat brain we have found appreciable amounts of A-protein which are associated primarily with the synaptosomal fractions, as isolated by differential centrifugation techniques. The enzyme activity associated with these particles is not sensitive to modification by α-lactalbumin unless it is first converted to a soluble protein with detergent treatment. This solubilized enzyme, from either rat or bovine brain is kinetically indistinguishable from the A-protein in bovine milk. It may readily be purified by affinity chromatography on columns of α-lactalbumin bound to sepharose.

Scientific Significance: The lactose synthetase system represents a novel type of evolutionary adaptation in which an enzyme which is normally used for glycoprotein and glycolipid biosynthesis (the A-protein) is modified to accomplish a new function, lactase biosynthesis, by complexing with another, non-catalytic protein, α-lactalbumin. Thus, the system is a clear example of how protein function can be modeified in a dramatic way by incorporation into more complex structures. Furthermore, the A-protein is known to be associated with membrane systems such as synaptosomes and liver cell membranes and may have an important role in membrane synthesis or function.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 35
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Protein Conformation and Limited Proteolysis

Previous Serial Number: Same

Principal Investigator: Werner A. Klee

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/8
Professional:	1/8
Other:	

Project Description:

Objectives: Although it is now a well established principle that the linear sequence of the amino acids of a polypeptide chain determines the nature of the characteristic structure (conformation) of proteins, the process whereby this transformation of two disensional information to a three dimensional structure occurs is not well understood. The ultimate goal of this project is to supply experimental data which may lead to some insight into this problem. A closely related question which we would like to help answer is: What is the nature of the conformational changes which take place in protein systems and what is the role of such structural flexibility in the functioning of macromolecular systems?

Methods Employed: One way of finding out something of the nature of the conformational determinants of proteins is to study the properties of smaller fragments of the molecules. We have prepared a number of peptides of overlapping sequence from three proteins by a combination of proteolytic and chemical cleavage, and are studying the conformational properties of these materials by physical methods which include spectropolarimetry, ultraviolet and nuclear magnetic spectroscopy, ultracentrifugation and titrimetry. These studies are

performed over a range of solvent conditions and temperatures and provide information on the conformation of these peptides in solution which can then be compared with the known structures of these materials when they are part of the intact protein in the crystalline state. We also study the nature of the conformational changes in which intact proteins participate on variation of the state of their environment or as a result of interactions with other kinds of molecules. This work uses the same kinds of physical methods described above in conjunction with some more subtle probes of structure such as limited proteolytic cleavage of exposed (and unstructured) regions.

Major Findings: We have studied in detail the conformational properties of a number of peptide fragments of ribonuclease, of insulin and of α-lactalbumin. The peptides chosen for study to date are all fragments containing the amino terminus of the protein and vary simply in the length of the rest of the polypeptide chain which remains attached. In this way a comparison of the properties of closely related peptides gives information about the structure determining role of successive regions of the polypeptide sequence. The results of this work to date are consistent with the hypothesis that those portions of the polypeptide chain of the proteins which are found to be in a helical region of the intact protein participate in a helix-like structure in solution as well. In isolation, these helices have rather different stability properties from those of the protein and may be more appropriately considered to be "nascent" helices rather than fully formed ones. Peptide segments which do not participate in a repeating structure in the intact protein appear to be essentially disordered in solution. An analysis of the published data from other laboratories with peptides derived from other proteins indicates thatthe phenomenon of "nascent helix" formation by fragments of helical regions of intact proteins may be a general one.

Our work with ribonuclease fragments had shown that the nascent helix near the amino-terminus of the molecule is stable only at low temperatures when fragments of short length are studied. The behavior of the nascent helix of the insulin B chain, (which corresponds to the amino terminus of proinsulin) is interesting in that it is stable only at high temperatures (37^{o}) and becomes disordered as the temperature is lowered. It thus appears that there may be more than one kind of nascent helix which is formed. It may be that the structure in the case of the insulin fragment is stabilized largely by hydrophobic interactions whereas that of the ribonuclease fragment is stabilized primarily by other kinds of forces.

The three dimensional structure of α-lactalbumin (see Project M-LGCB 18) has so far eluded solution by x-ray crystallographic methods. The amino acid sequence of this protein is, however, known to be very closely related to that of the well characterized protein antibiotic, lysozyme and it has been proposed that the conformation of the two proteins should therefore be similar. Studies of the physicochemical properties of the two proteins in this and other laboratories support this hypothesis. We have now succeeded in preparing mixed crystals of the two proteins which are stabilized primarily by salt linkages and appear to be composed of α-lactalbumin and lysozyme in the molar ratio of 1:2. It is possible that the availability of such a mixed crystal system will facilitate the x-ray analysis of α-lactalbumin structure.

Scientific Significance: These studies are providing a stock of techniques which may be generally useful in studying the nature of the determinants of protein structure. On the basis of our results it would appear that short range interactions, among near neighbor amino acid residues, control the nature of the protein structure to an important degree. The specific types of short range interactions involved are yet to be determined but it would appear that there may be at least two distinct ways of specifying helical structures.

Proposed Course of Research: We intend to continue these studies along the general lines discussed above.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 56
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Determination of the Effect of Small Viruses and Their Nucleic Acids On the Biochemistry of Living Organisms

Previous Serial Number: Same

Principal Investigator: Carl R. Merril

Other Investigators: T. Friedman, K. Krell, M. Geier, R. Yarkin and I. Prissovsky

Cooperating Units: None

Man Years:

Total: 5-1/2
Professional: 3-1/2
Other: 2

Project Description:

Objectives: An attempt to determine the genetic information contained within the nucleic acids of small "viruses" and their effect on various organisms.

Methods Employed: Biochemical techniques for purification and analysis of small viruses and nucleic acids, ultraviolet spectroscopy, density gradient ultracentrifugation, bacteriophage genetics, radioactive tracer techniques, mammalian tissue culture methods, studies with small mammals and developmental genetics.

Major Findings: 1. It has been possible to extend our observations on the function of bacteriophage carried genes in human cells to three new galactosemic cell lines, GM52, GM53, and GM54. All of these lines are not normally able to synthesize the enzyme UDP-gal transferase. The enzyme appearing after infection with λpgal virus behaves neither like the normal bacterial or normal human enzyme on preliminary enzyme inhibition studies.

2. In an effort to follow the expression of prokaryotic genes through a number of cell generations, it is important to work with cells capable of cloning. It has been found that human galactosemic fibroblasts are not capable of growing in Eagle's MEM containing galactose and dialyzed serum. We are presently investigating the possibility that galactosemic cells can be rescued in this medium by treatment with λpgal.

3. The fate of bacteriophage lambda in small mammals has been studied: a) as a model system for host virus interactions; b) to determine some of the parameters involved in attempts to do gene and/or enzyme therapy in mammals. The current studies have revealed that the spleen is engaged in a non-destructive capture of virus and may serve as a reservoir of antigen. Such an antigen reservoir may be necessary to illicit an immune response to a single antigen challenge.

4. Studies utilizing galactosemic cell lines have revealed that the rapid method of detecting galactosemic fetuses proposed by Hill and Puck based on the presumed inhibition of one of the pathways, galactolactone, is due to an artifact. It was found that galactolactone breaks down to galactonic acid and it is this acid which inhibits the galactosemic cells, rather than the specific blockage of a metabolic pathway which Hill and Puck first proposed. Unfortunately, galactosemic fibroblasts do not fulfill this criteria. Chinese hamster ovary cells and mouse L cells can be grown and cloned in chemically defined media. By omission of biotin the cells should not be capable of survival. It is hoped that such cells can be rescued by infection with a biotin transducing bacterial virus λpbio. By cloning rescued cells the permanence of the change can be studied.

Scientific Significance: The initial observation, that prokaryotic genes can function in eukaryotic systems, has been extended to further galactosemic cell lines, GM52, GM53 and GM54, indicating that the original results were not unique to the CCL-72 cell line. A number of workers have utilized similar techniques in plant cell studies, illustrating that plant cells can be altered by such viruses. This is especially significant because if one were able to construct a bacterial virus carrying the nitrogen fixation atmospheric nitrogen into organic nitrogenous products such as amino acids and proteins. The observation that bacterial viruses can produce proteins in mammalian cells also raises

the possibility that these viruses are directly or indirectly involved in human disease. It has already been well established that bacteriophage can indirectly cause human suffering by formation of toxins in bacterial hosts. With the demonstration of direct bacterial virus gene function in eukaryotes, it may be that some bacterial viruses act directly in the mammalian host cells. Mankind has lived in symbiosis with the bacterial E. coli in his large intestine for some time. It would be surprising in view of this relationship if there were not some viruses which could not live in both hosts. It may also be that some of these viruses are directly responsible for human disease.

Proposed Course of Research: To continue the study of these viruses and their interaction with other life forms, with emphasis on molecular level events.

Honors and Awards: None

Publications:

Friedman, T. B., Yarkin, R. J., and Merril, C. R.: Galactosemia and galactonolactone: further observations. Science 183: 764-765, 1974.

Merril, C. R., and Stanbro, H.: Intercellular gene transfer. Zeitschrift für Pflanzenphysiologie, in press.

Merril, C. R., Geier, M. R., and Trigg, M. E.: Transduction in mammalian cells. Excerpta Medica, in press.

Merril, C. R.: Bacteriophage interactions with higher organism. Trans. N. Y. Acad. Sci., in press.

Geier, M. R., Trigg, M. E., and Merril, C. R.: The fate of bacteriophage lambda in non-immune germfree mice. Nature 246: 221-223, 1973.

Friedman, T. B., and Merril, C. R.: Phage in serum. Proceedings of the Workshop on the Problems of Bacteriophage Contamination. Sponsored by: Bureau of Biologics, FDA, National Institute of Allergy and Infectious Diseases, NIH and National Cancer Institute, NIH. Held at Wilson Hall, NIH, Aug. 1, 1973, Sec. C, pp. 1-7.

Klingmuller, W., Krell, K., and Shimada, K.: Genetic analysis of bacteriophage lambda strains which transduce the genes for leucine biosynthesis. Molec. gen. Genet. 126: 1-6, 1973.

Geier, M. R., Trigg, M. E., and Merril, C. R.: A model system for the evaluation of the fate of phage in contaminated vaccines: Physiologic disposition of bacteriophage in mice. Proceedings of the Workshop on the Problems of Bacteriophage Contamination. Sponsored by: Bureau of Biologics, FDA, National Institute of Allergy and Infectious Diseases, NIH and National Cancer Institute, NIH. Held at Wilson Hall, NIH, Aug. 1, 1973, Sect. C, pp. 1-17.

Merril, C. R.: Effects of bacteriophage on eukaryotes. In Beers, R. F., and Tilghman, R. C. (Eds.): Cellular Modification and Genetic Transformation by Exogenous Nucleic Acids. Sixth International Symposium on Molecular Biology. Baltimore, Johns Hopkins Univ. Press, 1973, pp. 73-80.

Trigg, M. E., Geier, M. R., and Merril, C. R.: Letter to the Editor. N. Engl. J. Med. 289: 755, 1973.

Serial No. M - LGCB 58
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ribonuclease Specific for RNA-DNA Hybrids

Previous Serial Number: Same

Principal Investigator: Giulio L. Cantoni

Other Investigators: Peter K. Chiang

Cooperating Units: None

Man Years:

Total: 1/2
Professional: 1/2
Other:

Project Description:

Objectives: To gain further knowledge about the function, intracellular distribution and molecular activity of ribonuclease H, an enzyme that specifically cleaves the RNA part of an RNA-DNA hybrid.

Methods Employed: Standard methods of biochemistry.

Major Findings: We have continued our studies on calf thymus ribonuclease H that has been highly purified by Haberkern and Cantoni. In addition, we are in the process of comparing the properties of ribonuclease H from calf thymus with that from other tissues, such as white blood cells, E. coli, and yeast. S-Adenosylmethionine at 0.5 → 1 mM concentration is a potent inhibitor of ribonuclease H. The significance and specificity of this observation is not yet clear. Also, spermidine, at very low concentration has been found to stimulate the enzyme activity, perhaps by influencing the conformation of the DNA-RNA hybrid.

Scientific Significance: Spermidine is known to play a role in the control of nucleic acid synthesis. It is interesting to speculate that this might involve a stimulation of DNA synthesis by hydrolysis of its RNA primer, perhaps through

stimulation of ribonuclease H. Experimental verification of this speculation will be very complex and we are attempting to devise methods to approach the problem.

Proposed Course of Research: Further studies of the enzyme and its role are planned. A hypothesis linking ribonuclease H and the post transcriptional control of messenger RNA is being elaborated and will in due course be subject to experimental scrutiny.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 60
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the Biochemical Basis of Narcotic Drug Action

Previous Serial Number: None

Principal Investigator: Werner A. Klee

Other Investigators: Marshall Nirenberg, Shail Sharma, Everette May, Arthur Jacobson

Cooperating Units: Lab. of Biochemical Genetics, NHLI
Section on Medicinal Chemistry, Lab. of Chemistry, NIAMDD

Man Years:

Total:	1-1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives: To study the interaction of morphine-like compounds with receptor sites in the nervous system and the significance of such interactions to the function of the nervous system at the biochemical level.

Methods Employed: Subcellular fractionation of brain and other tissues and cells of neuronal character. Tissue culture of neuroblastoma cells and derivatives of such cells. Drug binding measurements with highly radioactive narcotics and by competition methods. Estimations of changes in cellular metabolism and function using standard and specially modified biochemical and electrophysiological techniques.

Major Findings: We have in the past year refined our binding assays so as to clearly demonstrate the stereospecific and saturable binding of ^{3}H-dihydromorphine to membrane fragments of rat brain cells. A large number of other narcotics have been shown to compete for these binding sites with affinities which are in general accord with their known analgesic or

antagonist activities in animals and man. Rats made dependent on and tolerant to morphine by pellet implantation do not appreciably inverse the number of narcotic receptor sites.

In a parallel study we have examined a number of clonal neuroblastoma cells for the presence of narcotic receptors. These cell lines are of great interest since many of them are known to be exclusively adrenergic or cholinergic and since a number of regulatory mechanisms of neurotransmitter synthesis and morphological differentiation have been described for them. None of the neuroblastoma cells examined showed the presence of narcotic receptors within the sensitivity of our assay. On the other hand, a single clonal cell line (N 108-15) formed by fusion and hybridization of a mouse neuroblastoma cell line (N18T62) and a rat glioma cell line (C_6Bul) does clearly express specific narcotic receptors which are almost identical to those which we study in the rat brain. Other somatic cell hybrids show little or no specific narcotic binding. The NG108-15 cell line produces and, stores in vesicles, large amounts of acetyl choline but no catecholamines. Other cell lines which also are cholinergic do not bind narcotics in our assays. Thus, there is no obligatory relationship between neurotransmitter type and narcotic receptors. The receptors of NG108-15 are localized on the cell surface membrane and exhibit the same density in a sucrose gradient as do those of the brain. Also, the relative binding affinity of a series of narcotics is the same with the receptors from the two sources although there are small differences in the absolute magnitudes.

We have found that the prostaglandin E stimulated adenyl cyclose activity of homogenates of NG108-15 is inhibited by narcotics as has been recently reported for brain by Collier and Roy. This interaction may represent an early step in the mechanism of action of the narcotics and is being actively pursued.

Scientific Significance: In the past year or so there has been a quantum jump of progress in the study of the mechanism of action of narcotics due largely to the development, in a number of laboratories here and abroad, of methods for the study of narcotic-receptor interactions in vitro. It is now possible to determine the number of receptors directly and thus test some theories of drug dependence and tolerance, as well as to gain insight into the basic mechanism of narcotic analgesia. The discovery of a tissue culture system with receptor properties similar to brain provides a homogeneous

population of cells with which to study the narcotics. With this system it is reasonable to hope that each step between the narcotic-receptor interaction and the final effect on the neuronal function can ultimately be unravelled.

Proposed Course of Research: We intend to continue our studies as outlined above.

Honors and Awards:

A paper on "Neuronal Cells in Tissue Culture With Narcotic Receptor Properties," was chosen for presentation at the 36th Annual Meeting of the Committee on Problems of Drug Dependence of the National Research Council.

Publications:

Klee, W. A., and Streaty, R. A.: Narcotic receptor sites in morphine dependent rats. Nature 248: 61-63, 1974.

Serial No. M - LGCB 61
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Muscle Differentiation in Tissue Culture

Previous Serial Number: Same

Principal Investigator: Giulio L. Cantoni

Other Investigators: Sigfrido Scarpa and B. William Uhlendorf

Cooperating Units: None

Man Years:

Total:	3-1/2
Professional:	2-1/2
Other:	1

Project Description:

Objectives: Embryonic skeletal muscle culture system in vitro has been recognized as a potential model of cellular differentiation. The principal differentiative event is the fusion of mononucleated myoblasts into multinucleated myofibers, that then go on to form fully differentiated striated myotubules. The mechanism of myoblast fusion and the factors controlling it are not adequately understood.

Methods Employed: Embryonic muscle is cultured in vitro and the degree of differentiation is evaluated by a combination of cytological and biochemical tests. The influence of various components of the culture medium on the course of differentiation is evaluated.

Major Findings and Scientific Significance: Most of our efforts in the past year have been devoted to the development of methodology suitable for the attack of the objective outlined above. Earlier work in this laboratory, as well as in other laboratories, has utilized primary culture of cells derived from embryonic muscle as a source of myoblast. While this system is advantageous in that muscle fusion can be detected in a relatively few days (4-8), it suffers from one important disadvantage from the

biochemical point of view: it consists of a mixed population of at least two principal cell types with different phenotypes and biochemical properties: a) fibroblasts, and b) dividing mononucleated myoblasts. This latter group usually predominates quantitatively by three or four to one. This heterogeneity is clearly disadvantageous and we turned our attention to muscle cell lines, originally derived from primary cultures of embryonic muscle, but maintained in culture for long periods of time. Unfortunately, the cell line system is much slower, but it offers at this stage of our investigation overriding advantages. We originally obtained such cell lines through the kindness of Dr. M. Nirenberg, who in turn had received them from Dr. David Yaffe of Israel. The lines, as such, are incompletely characterized. From these cell lines we have obtained several sublines, starting from a single cell by cloning techniques. We now have several fusing and non-fusing myoblast lines consisting of a highly homogeneous cell population. As noted above, measured in time units, progress is very slow owing to the inherent characteristis of cells in culture. Thus, with a division time of approximately 24 hours, it takes approximately 20-30 days (that is 20-30 divisions) to bring a single cell to a number sufficient to work with, 10^6-10^7 cells.

In order to develop a "differentiation index," it is essential to be able to distinguish and count the nuclei in mononucleated cells and those in polynucleated muscle fibers. We are making substantial progress toward this goal through the development of a quantitative technique for nuclear count with a sophisticated particle counter system. Nuclear count will be correlated with measurement of specific muscle enzyme (creatine phosphokinase and the ability to synthesize DNA. Radioautography and karyotype studies are beginning to yield very interesting data.

While our efforts have been primarily methodological, we have made a number of interesting observations that we are in the process of following up. We noticed, for instance, that the chromosome number of fusing and non-fusing clonal cell lines (derived from the same parent line) is different. This observation will need to be extended, but it is very provacative. Patterns of DNA synthesis seems to be emerging from our radioautographic studies. In summary, we have made good progress in developing the methodology necessary to study the biochemical parameters and mechanisms of cellular differentiation of myoblasts <u>in vitro</u>.

Proposed Course of Research: We are in the early phases of this project and there are many options to us for future studies. Our course will be determined by the outcome of the exploratory experiments under way.

Honors and Awards: None

Publications: None

Serial No. M - LGCB 64
1. Laboratory of General and Comparative Biochemistry
2. Section on Proteins
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the Characteristics and Regulation of Adenosylhomocysteine

Previous Serial Number: None

Principal Investigator: Giulio L. Cantoni

Other Investigators: Henry H. Richards

Cooperating Units: None

Man Years:

Total:	1-1/4
Professional:	1/4
Other:	1

Project Description:

Objectives and Scientific Significance: S-Adenosylhomocysteine, the product of methyltransfer reaction from S-adenosylmethionine, was discovered and characterized by Cantoni and his collaborators. The only pathway known for further metabolism of adenosylhomocysteine is the reaction

$$\text{ASR} \rightleftharpoons \text{adenosine} + \text{homocysteine}$$

catalyzed by adenosylhomocysteinase, an enzyme studied in this laboratory several years ago. The reaction is reversible with the equilibrium strongly in the direction of ASR synthesis. ASR is a powerful inhibitor of many of the methyl transfer reactions which utilize adenosylmethionine as the methyl donor, and therefore the tissue levels of ASR become an important regulatory element in the biosynthesis of methylated compounds, such as neurohormone or neurohormone precursors (methylated catecholamines, methylhistamines, methylated indolamines and choline), proteins (myosin, histones, encephalitogenic protein), and nucleic acids (tRNA, mRNA, DNA). ASR hydrolyase has been characterized only very incompletely in the past. Further work on the

enzyme, its tissue and intracellular distribution, its regulation and the effect of analogues and/or inhibitors will be of interest. This approach will complement our studies on a series of AMe and ASR analogues being synthesized for us under Contract #HSM-42-73-8 at the University of Kansas.

Methods Employed and Major Findings: A new, very sensitive assay method has been developed. With this assay we are proceeding to purify the ASR hydrolase from beef liver, and will next study the enzyme distribution in different tissue and species, especially in bacteria, such as E. coli, Salmonella, and other enterobacteria where methionine biosynthetic regulatory mutants may lack the enzyme. Also, with the purified enzyme, we plan to develop a general method for the study of methyltransfer reactions from adenosylmethionine that will enable us to screen rapidly a large number of adenosylmethionine analogues as potential inhibitors of specific and biologically important transmethylation reactions.

Proposed Course of Research: This project may be considered a continuation of a project carried out by de la Haba and Cantoni. It complements other studies in progress or being planned and will extend over the next couple of years at least. Adenosylmethionine and its analogues are reported as useful pharmacological agents in the treatment of some human pathological states. While it is too early to assess the validity of these reports, the significance of this and related projects to human disease should not be discounted.

Honors and Awards:

Invited, as principal lecturer, to attend the International Symposium on S-Adenosylmethionine, sponsored by Academia Nazionale dei Lincei, Rome, Italy, May 21-26, 1974.

Publications: None

Serial No. M - LGCB 43
1. Laboratory of General and Comparative Biochemistry
2. Section on Alkaloid Biosynthesis
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Homocystinuria: Methionine Metabolism in Mammals

Previous Serial Number: Same

Principal Investigator: S. Harvey Mudd, M.D.

Other Investigators: B. W. Uhlendorf, Ph.D.

Cooperating Units: None

Man Years:

Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: The objectives of this project are to obtain a detailed knowledge of the biochemistry of sulfur-containing compounds in the mammalian body, with emphasis on the transsulfuration pathway. Special attention will be focused on the regulatory devices which control this area of metabolism.

Methods Employed: Most of the methods employed have been presented in detail in previous Annual Reports. In general, standard methods of enzyme purification and assay have been employed. Automated amino acid analysis has been used extensively. The techniques used for growth of cells in tissue culture are the usual ones. Methods used during the past year have all been described in previous Annual Reports.

Major Findings: Our chief preoccupation for the past year with respect to this project has been to bring to completion the studies of nitrogen balance and sulfur excretion previously described in a preliminary way. Several points have emerged rather clearly from the completed studies: (a) A daily intake of 650-680 mg. of methionine is adequate to maintain healthy young adult males in the zone of nitrogen equilibrium. This is comparable to the amounts previously reported by others to be required by young adult females, but is far less than the 1100 mg. specified by Rose and co-workers as needed to maintain "distinctly positive" nitrogen balances in young men. It appears likely that this discrepancy is due to

a difference in the "end-points" used in the two series of studies, and that, contrary to previous conclusions, there is indeed little, if any, difference in the sulfur amino acid requirement of men and women. (b) Slightly more methionine may be required to maintain sulfur equilibrium than to maintain nitrogen equilibrium. (c) Cystathionine synthase deficient individuals with no residual activity of this enzyme detected in fibroblasts require 300 mg cystine daily to maintain nitrogen equilibrium and, possibly, 500 mg to maintain sulfur equilibrium. (d) Cystathionine synthase deficient individuals with a 1-2% residual activity of this enzyme according to in vitro measurements (for example, B_6-responsive patients) have no demonstrable requirement for exogenous cyst(e)ine. On intakes of this amino acid as low as 4-12 mg, such subjects remained in nitrogen equilibrium, lost about as much sulfur as control subjects, and raised their plasma cyst(e)ine concentrations. These findings suggest that such cystathionine synthase deficient individuals must form endogenously at least 300-500 mg (2.5 - 4.1 millimoles) cysteine daily. Presumably this conversion is catalyzed by the residual cystathionine synthase activity. If this residual activity were increased 2-4 fold, the patients would become capable of daily converting at least 10 millimoles of homocysteine to cystathionine, an amount approximately equal to the normal daily intake. Thus, our previous working hypothesis, that a relatively small increase in residual cystathionine synthase activity should be sufficient to account for the B_6-response, is strongly confirmed. (e) A parallel conclusion may be applicable to the single B_6-responsive cystathioninuric patient available for study. It is suggested this patient has residual cystathionase activity which is enhanced by B_6 treatment.

Scientific Significance: The significance of these results is indicated in the section above. At present, at least 20 genetically determined human diseases are known to be responsive to one of 7 vitamins. This list is rapidly expanding. Our studies of the mechanism of the B_6-responses in cystathionine synthase and cystathionase deficiencies permit further understanding of how these responses occur, and rational extension of this approach to a variety of additional diseases. The results are hopeful in suggesting that this type of "genetic engineering" is at present feasible and likely to be truly beneficial.

Proposed Course of Research: Unfortunately, loss of our clinical collaborators due to changes within the NIAMDD and severe curtailment in the availability of Dr. Uhlendorf for this project are forcing us to phase out most of our work on metabolic diseases. As one example, we have been unable to carry out the logical extension of the sort of study described here to other areas. The future of this project appears now to depend upon the outcome of attempts to find suitable collaborators within other Institutes, and on whether or not increased assistance with tissue culture can be found. For these reasons, we have repeatedly during the past year been forced to turn down requests for collaborative studies with workers in Universities.

Honors and Awards:

At the invitation of the Royal College of Pathologists, delivered a lecture, "Vitamin-responsive genetic disease", at the Symposium on Molecular Variant in Disease, London, England, February 14, 1974.

Publications:

Mudd, S. H.: Homocystinuria and homocysteine metabolism. In Nyhan, W. L. (ed.): Amino Acid Metabolism and Genetic Variation. New York, New York, McGraw-Hill Book Co., 1974 (in press).

Mudd, S. H.: Vitamin-responsive genetic disease. J. Chem. Path., 1974 (in press).

Dillon, M. J., England, J. M., Gompertz, D., Goodey, P. A., Grant, D. B., Hussein, H. A-A., Linnell, J. C., Matthews, D. M., Mudd, S. H., Newns, G. H., Seakins, J. W. T., Uhlendorf, B. W., and Wise, I. J.: Mental retardation, megaloblastic anaemia, methylmalonicaciduria, and abnormal homocysteine metabolism due to an error in B_{12} metabolism. Clinical Science and Molecular Medicine, 1974 (in press).

Mudd, S. H. and Freeman, J. M.: $N^{5, 10}$-Methylemetetrahydrofolate reductase deficiency and schizophrenia: a working hypothesis. In Matthysse, S. (ed.): "Conference on Catecholamines and the Enzymes in the Neuropathology of Schrizophrenia"; Proc., 1974 (in press).

Serial No. M - LGCB 48
1. Laboratory of General and Comparative Biochemistry
2. Section on Alkaloid Biosynthesis
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Transsulfuration in Higher Plants

Previous Serial Number: Same

Principal Investigators: John Giovanelli, Ph.D. and Anne H. Datko, Ph.D.

Other Investigators: S. Harvey Mudd, M.D.

Cooperating Units: None

Man Years:

Total: 2-1/6
Professional: 2-1/6
Other: 0

Project Description:

Objectives: Previous work has shown that extracts of spinach and other higher plants catalyze the synthesis of homocysteine (and methionine) by two main pathways - a direct sulfhydration pathway and a transsulfuration pathway. The long-range objective of the project is to determine the relative physiological significance and the mechanisms of control of these pathways. The problem is being attacked along the following lines:

(1) A study of the enzymes involved in the two pathways, and the in vivo concentration of the substrates for these enzymes.
(2) In vivo evaluation of the relative physiological significance of the two pathways by use of inhibitors that specifically block one of the pathways.
(3) Physiological and biochemical studies with algae (wild type), and algal mutants in which the biosynthesis of homocysteine and methionine is affected.

Methods Employed: The main methods employed have been described in previous reports. Most of the procedures for isolation and characterization of cystathionine require its oxidation by hydrogen peroxide or performic acid. The products of these oxidations were characterized as the sulfoxides (presumably the two diastereoisomers) and the sulfone of cystathionine. Conditions were devised in which the major products of oxidation of cystathionine by hydrogen peroxide were the sulfoxides, and by performic acid was the sulfone. A chemo-stat designed for the steady-

state photoautotrophic culture of Chlorella was constructed. The apparatus has performed well in experiments in which Chlorella was grown in the presence of limiting sulfate-^{35}S, and promises to be a very versatile tool for the proposed physiological and biochemical studies of Chlorella.

Major Findings: (1) We have previously reported that no generally distributed plant enzyme could be detected that catalyzed the synthesis of either O-malonyl-, O-succinyl-, O-oxalyl- or O-acetyl-homoserine. This key finding has been confirmed with assays that are approximately ten times more sensitive than these previously used, and with a wide phylogenetic range of plants.

(2) Enzymic synthesis of O-phosphohomoserine from homoserine and ATP has previously been reported in a wide range of plant tissues. This enzyme activity will be the subject of intense study at the earliest opportunity, since it is probably a control site in the synthesis of methionine and threonine. Preliminary data have already suggested that homoserine kinase of plants may exist in at least two different forms with different cellular locations.

(3) A sensitive and specific assay for cystathionine was developed. The method utilizes the ability of a crude extract of Bacillus subtilis to catalyze the exchange of cysteine-^{14}C into cystathionine. The method has been successfully applied to the assay of cystathionine in plant tissues and human urine. Cystathionine was demonstrated to be widely distributed in plant tissues at concentrations ranging from 0.04 to 2.6 nmoles/g fresh weight. The presence of cystathionine in all of the tissues studied is further evidence for operation of the transsulfuration pathway in green plants.

(4) Experiments are in progress to assess the relative rates of flow of ^{35}S through the direct sulfhydration and transsulfuration pathways during the steady-state growth of Chlorella.

Scientific Significance: Discussed under individual headings above.

Proposed Course of Research: (1) Studies on homoserine esterification in crude extracts of a range of plant tissues suggest that O-phosphohomoserine synthesis is a major pathway for homoserine activation in plants. Direct confirmation of this suggestion has been provided for cystathionine synthesis, in which O-phosphohomoserine is the major, perhaps the sole, physiological α-aminobutyryl donor. It is proposed to examine what forms of activated homoserine are the specific precursors of threonine, and of homocysteine in the direct sulfhydration pathway.

(2) Evaluation of the relative significance of the two pathways will continue along two main lines: (a) Correlation of physiological states and levels of enzymes and substrates of S-amino acid biosynthesis. (b) Determination of the initial kinetics of incorporation of sulfate-^{35}S into

S-amino acids, in the absence and presence of rhizobitoxine, a specific inhibitor of β-cystathionase. Chlorella grown under steady-state conditions will be used in each of these approaches.

Honors and Awards: None.

Publications:

Datko, A. H., Giovanelli, J. and Mudd, S. H.: Homocysteine biosynthesis in green plants. O-Phosphorylhomoserine as the physiological substrate for cystathionine γ-synthase. J. Biol. Chem. 249: 1139-1155, 1974.

Serial No. M-NB-1
1. Lab of Neurobiology
2.
3. Bethesda, Maryland,
Woods Hole, Mass., and
Sapporo, Japan

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Analysis of the macromolecular structure of nerve membranes during excitation

Previous Serial Number: Same

Principal Investigators: Ichiji Tasaki, M.D., Kenneth Sisco, M.D., Akira Warashina, Ph.D., and Ronald Sandlin

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass., and Hokkaido University, Sapporo, Japan

Man Years

Total:	4.1
Professional:	3.5
Other:	0.6

Project Description

Objective: During the past year the research activity of the group of investigators was directed toward elucidating the physicochemical basis of the extrinsic fluorescence signals in crab and squid nerve fibers. The spectrum of the portion of the fluorescent light deriving from aminonaphthalene-sulfonate compounds incorporated into the axonal membrane was determined with a special high-time resolution spectrofluorometer constructed in this laboratory. The spectrum measured in the resting state of the axon was not very different from that of those fluorochromes bound non-covalently to various commercially available biopolymers (lipids and proteins). The portion of light contributing to a small change in the fluorescence intensity (i.e., to fluorescence spikes) was found to have a very sharp and narrow spectrum which could not be encountered when these fluorochromes were incorporated into any of the biopolymers. Elucidation of the physicochemical basis of this unusual spectrum was expected to throw considerable light on the process of nerve excitation occurring in the nerve membrane.

Methods Employed: Using the fluorescence techniques described in the previous annual report, extensive spectral analyses of the fluorescence signals were undertaken using either H_2O or D_2O containing sea water. In addition to *in vitro* studies with a Perkin-Elmer spectrofluorometer, nanosecond decay studies (Ortec) were also done of dye solutions as well as the stained squid axon.

Major Findings: Extensive in vitro studies were made of the physicochemical factors influencing the intensity of the spectrum of 2-p-toluidinylnaphthalene-6-sulfonate (2,6-TNS). The conclusions drawn from these studies are as follows:

(1) The emission spectrum of 2,6-TNS is strongly affected by the solvent polarity, as shown previously by McClure and Edelman.

(2) Chemical quenching of 2,6-TNS influences the fluorescence intensity without affecting the emission spectrum.

(3) A change in the number of bound 2,6-TNS molecules alters the fluorescence intensity again without being accompanied by a shift in the emission maximum of the spectrum.

(4) A change in the viscosity of the medium can affect both the emission spectrum and the fluorescence intensity.

Significance: Based on the results of these in vitro studies, it was concluded that fluorescence signals in squid and crab nerve fibers are produced by a change in the polarity of the microenvironment around the fluorescent probe molecules in the axon membrane. A full account of these studies is published in an article entitled, "Spectral analyses of extrinsic fluorescence of the nerve membrane labelled with aminonaphthalene derivatives" by Tasaki, Carbone, Sisco and Singer, published in Biochim. Biophys. Acta (1973).

In addition to these spectral studies, Drs. Carbone, Sisco and Warashina examined the role of water molecules in the process of nerve excitation. The effect of substitution of water in the artificial saline solution for heavy water was found to affect the intensity of the fluorescent light emitted by nerve fibers labelled with 2,6-TNS. It was concluded that there is direct involvement of the water molecules in the process of nerve excitation. The details of these studies are described in a manuscript submitted for publication in J. Memb. Biology.

In collaboration with Dr. Harold Gainer of the National Institute of Child Health and Human Development, Dr. Tasaki conducted an extensive study of the process by which amino acids are incorporated into proteins in internally perfused squid giant axons. During his field trip to Sapporo, Japan, Dr. Tasaki collaborated with Drs. Kobatake and Inoue of Hokkaido University extending previous physicochemical analyses of the conformational changes in the axon membrane.

Proposed Course: Further experiments using spectral analysis of extrinsic fluorescence will be done to elucidate the physicochemical basis of nerve excitation.

Honors and Awards:

Dr. Tasaki was invited to attend and present papers at a number of symposia, including the Advanced Study Institute, Université de Rouen, France, the University of Bern, Switzerland, the "Orbis Scientiae" sponsored by the Center for Theoretical Studies, University of Miami, and the Neural Membranes symposium of the 5th Annual Meeting of the American Society for Neurochemistry.

In addition, Dr. Tasaki was invited by the Japan Society for the Promotion of Science to visit Japan as a Visiting Professor for 70 days. While in Japan he gave a lecture to the Group of Young Researchers of Biophysics, University of Tokyo, and lectured at the Hokkaido University Special Lecture Series for Graduate Students.

Publications:

Sato, H., Tasaki, I., Carbone, E. and Hallett, M.: Changes in axon birefringence associated with excitation: Implications for the structure of the axon membrane. J. Mechanochem. Cell Motility 2: 209-217, 1973.

Tasaki, I.: Energy transduction in the nerve membrane, and studies of excitation processes with extrinsic fluorescence probes. Ann. N.Y. Acad. Sci. 227: 247-268, 1974.

Tasaki, I., Carbone, E., Sisco, K. and Singer, I.: Spectral analyses of extrinsic fluorescence of the nerve membrane labeled with aminonaphthalene derivatives. Biochim. Biophys. Acta 323: 220-233, 1973.

Serial No. M-NB-2
1. Lab of Neurobiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Sensory-motor integration in the primate visual system

Previous Serial Number: Same

Principal Investigators: Robert H. Wurtz, Ph.D., David L. Robinson, Ph.D., Charles W. Mohler, M.D.,Ph.D. and Joan Baizer, Ph.D.

Man Years

Total:	6.5
Professional:	4.0
Other:	2.5

Project Description

Objective: In the past year we have compared how the two branches of the primate visual system relate to selection of visual stimuli for the guidance of eye or the triggering of arm movement. Through these studies we hope to gain a better understanding of how the nervous system functions in visually initiated behavior.

Methods Employed: The discharge of single brain cells has been recorded in awake monkeys (Macaca mulatta) trained to do several visually related tasks. The monkey sat in a chair facing a screen and fixated on a point of light for several seconds in order to obtain a reward. During this fixation period, a second spot of light was projected onto the screen and used to determine the area of the visual field in which the spot modified the discharge of the cell (the visual receptive field of the cell). Rapid eye movements were elicited by turning off one fixation point and turning on another point thereby inducing an eye movement from one spot to the other. An on-line digital computer was used to control the monkey behavior and display the single cell data while the experiment was in progress.

Major Findings: We have previously reported that cells in the brainstem branch of the visual system, the superior colliculus, responded differently when the monkey shifted its attention to the visual stimulus which activated the cells. We found that when the monkey prepared to make a rapid eye movement to the stimulus, cells in the superficial layers of the superior colliculus discharged more vigorously than when the monkey was merely fixating and presumably not attending to the stimulus. Furthermore, we found that the response of these cells was enhanced with eye movements made to the area of the stimulus, but not with eye movements made to other parts of the

visual field. This response enhancement is therefore a possible neural correlate of selective attention. In the other branch of the visual system, the striate cortex, we have now found that such selective enhancement does not occur. Here eye movements to the visual stimulus occasionally was associated with an enhanced response of the cell, but the enhancement was not selective -- it occurred with eye movements to all parts of the visual field. The enhancement of response in the cortex is therefore not selective as in the superior colliculus but rather general and may be related to level of arousal of the monkey rather than selective attention.

So far we had looked for an enhanced response of the brain cells when the monkey used the stimulus driving the cell as a target for an eye movement. We next wanted to know if we would also find the same effect if the monkey responded to the stimulus with his hand rather than with his eye. We therefore designed an experiment which required the monkey not make an eye movement to the stimulus but just to remove his hand from a bar when the stimulus changed. We found that the cells in the superior colliculus did not show an enhancement of response with hand movement comparable to that seen with eye movement. While this experiment does not prove that the enhancement is specifically related to eye movements, it does show that the type of response made by the monkey does alter the processing of visual stimuli at a very early stage of the visual system. The result also indicates that the response of the cells may be more related to the response made to the stimulus and thus not be closely correlated with pure stimulus selection usually associated with attention. No enhancement effect with either eye or hand was seen in striate cortex neurons.

The cells in the intermediate layers we had previously found to respond before eye movements. The cell discharges were associated with eye movements to one area of the visual field and this area moved with the retinal map as the eye moved. But the eye must be guided to points in the real spatial world and at some point a transition between the retinotopic and the spatial map seems likely to occur. We looked for such a transition in deeper layers of the superior colliculus and could find none. Thus we have found that the superior colliculus both in its visual sensory cells and in its eye movement related cells,is similar to the striate cortex; both are tied to a retinotopic map.

Finally, we determined how damage of striate cortex cells and superior colliculus cells would influence the detection of small spots of light and the guidance of rapid eye movements to the small spots of light. Lesion of the colliculus alone did not alter the detection of spots of light nor, as we have found previously, did the lesion alter significantly the guidance of eye movements to the spot of light. Lesion of striate cortex disrupted both of these functions but the disruption largely disappeared within six weeks. However, if both the striate cortex and the superior colliculus were damaged, the monkey became functionally blind in our tests; he could neither detect a spot nor could he saccade accurately to it. Thus in both of these tasks, the striate cortex and the superior colliculus act as parallel pathways -- after recovery the presence of either pathway permits the animal to perform the task.

In addition, since the deficit found showed up for both detection of the spot of light as well as guidance of rapid eye movements to the spot, it is clear we are dealing with a sensory deficit not any deficit in the eye movement system alone.

Significance: One of the major ways we have of discovering brain function is by comparing how one area is related to behavior in comparison to another area of the brain. We have tried to expand our knowledge of the nervous system by using this method on the visual input pathways to the brain. We find that in some ways the two pathways -- one to the cerebral cortex and one to the midbrain -- are similar. Both maintain a retinotopic organization; both are capable of providing the sensory information for detection of light and for the guidance of eye movements to the spots of light. We have previously reported a major difference between these pathways: the striate cortex cells require precisely oriented, shaped, and positioned stimuli to be activated; the superior colliculus cells require only spots of light in a comparatively large part of the visual field to be activated. We now have found that it is the superior colliculus that appears to be concerned with selection of visual stimuli while the striate cortex is unaffected by the use the animal makes of the stimulus. The tentative picture that emerges from our experiments is that of a division of labor between cerebral cortex and brainstem at these early stages of processing. The cortex does the fine analysis of the stimulus; the brainstem selects and emphasizes which stimulus is important.

Proposed Course: We plan to study the two branches of the visual system further and to search for the possible integration of their functions at some subsequent stage of processing. We intend to explore successive stages of cerebral cortex to determine what type of integration occurs between the striate cortex and superior colliculus type of visual processing. We also intend to pursue behavioral experiments to try to determine which of these brain structures is related to different modes of visual processing.

Honors and Awards:

Dr. Wurtz was invited to present a paper at the International Symposium on "Motor aspects of behaviour and programmed nervous activities" in Marseille, France; to participate in a Work Session sponsored by the Neurosciences Research Program in Brookline, Mass.; and to participate in a meeting on "Eye movements and psychological processes" sponsored by the Behavioral Research Directorate of the U.S. Army Human Engineering Laboratory in Princeton, N.J.

Publications:

Mohler, C.W., Goldberg, M.E. and Wurtz, R.H.: Visual receptive fields of frontal eye field neurons. Brain Research 61: 385-389, 1973.

Robinson, D.L. and Jarvis, C.D.: Superior colliculus neurons studied during head and eye movements of the behaving monkey. J. Neurophysiol.37: 1974 (in press).

Wurtz, R.H. and Mohler, C.W.: Selection of visual targets for the initiation of saccadic eye movements. Brain Research 71: 1974 (in press).

Serial No. M-NB-3
1. Lab of Neurobiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Characterization and determination of protein invertebrates

Previous Serial Number: None

Principal Investigators: Robert H. Davis, Ph.D. and Kenneth Sisco, M.D.

Man Years

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description

Objective: During the past decade there have been few studies of the biochemistry of protein synthesis in the nervous system of invertebrates. Invertebrates offer a model system for study since no myelin surrounds the nerve bundles. Our objective was to study the incorporation rate of leucine into the soluble proteins of nerve bundles of lobster both peripheral and central. In addition, we hoped to determine the number of soluble proteins present in this system and the incorporation rate of several individual proteins.

Methods Employed: Polyacrylamine gel electrophoresis is being utilized to determine and separate the soluble proteins. With this technique the incorporation rate of individual proteins may also be determined. Liquid scintillation counting is the method of choice for counting the incorporated labeled precursor. Autoradiography is used to study the morphology of protein synthesis in the lobster nervous system.

Major Findings: We have determined the rate of protein incorporation both *in vivo* and *in vitro* for central and peripheral nervous systems in the lobster. The central nervous system incorporated counts *in vitro* at a much higher rate (60,000 c.p.m./mg protein) than peripheral (10,000 c.p.m./mg protein). The Schwann cells were particularly active in protein incorporation as evidenced by autoradiography. Gel electrophoresis confirmed the homogenous nature of the soluble proteins as reported by Fernandez. However, the number of bands evident on the gels were 28 as compared to 8 previously reported. In addition, the individual bands for ganglia contained a very high rate of incorporation and was the highest of any bands studied.

Significance: These findings may reflect an important role for the chwann cells in the maintenance of axons, and possibly nerve regulation.

Proposed Course: The intermediate metabolism of Schwann cells and ganglia ill be studied in respect to protein synthesis.

Serial No. M-NC-1
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Conversion of Phenylalanine to Tyrosine

Previous Serial Number: M-NC-1

Principal Investigator: Seymour Kaufman

Other Investigators: S. Milstien, J.-P. Abita

Man Years:

Total	3.3
Professional	2.3
Other	1.0

Project Description:

Objectives: The oxidation of foodstuffs, which ultimately supplies the energy for all cellular functions, can be effected by two completely different processes. Organic compounds can either be oxidized by dehydrogenation, the removal of hydrogen, or by oxygenation, the addition of oxygen to the substrate molecule. The first type of reaction predominates in carbohydrate and fat metabolism. The mechanism of this kind of oxidation has been the subject of investigation for 50 years and is now quite well understood. Oxygenation reactions are encountered mainly in the cellular catabolism of ring compounds such as aromatic and heterocyclic amino acids, hormones and drugs. In contrast to hydrogenations, the mechanism of oxygenation reactions has remained obscure. The conversion of phenylalanine to tyrosine is an oxidation reaction of this type and the goal of this study is to gain insight into the mechanisms of this kind of oxidation reaction.

Methods Employed: Standard method of protein purification, including salt and ethanol fractionation, ion-exchange chromatography and sephadex-gel filtration, have been used. The following methods of protein analysis have been used to characterize the hydroxylase: Sucrose-density ultracentrifugation and polyacrylamide gel electrophoresis.

Major Findings: The finding, described in last year's report, that there is detectable phenylalanine hydroxylase (PAH) in a liver sample from a patient with classical PKU has led to a major change in the direction taken in this area of research. That finding emphasized the importance of studying ways in which PAH activity can be increased in vivo. An increase of significant magnitude would have obvious therapeutic implications.

In order to assess physiological manipulations that are designed to enhance PAH activity, a reliable in vivo assay for the enzyme was needed. Ideally, the assay should be applicable to humans as well as to animals. A suitable assay has been developed which is based on the release of deuterium from ring-deuterated phenylalanine after administration of the latter compound to the animal. The D_2O in the blood is quantitatively determined by infra red measurements. The assay is sensitive enough to detect the 25% greater activity of phenylalanine hydroxylase in male rats as compared to females. Since the assay involves the use of relatively small amounts of the stable isotope, deuterium, it should be suitable for use in humans.

As an adjunct to the above method, we have also developed an assay for phenylalanine hydroxylase in liver slices. The results obtained with the in vivo assay (described above) and the slice assay are in excellent agreement. Preliminary results indicate that it is possible to stimulate the activity of PAH by administration of certain pterin cofactors to rats, or by addition of the pterin to the medium in which the liver slices are suspended.

Scientific Significance: There are two applications of the in vivo assay for PAH: a) to follow in vivo manipulations designed to activate PAH in experimental animals, and b) to measure PAH activity in patients with PKU, in those with hyperphenylalaninemia, and in individuals who are PKU heterozygotes. It is important that these three conditions be reliably distinguished early in life (within the first two to three weeks after birth) since the diagnosis will determine whether the suspect infant will be treated, i.e., fed a low-phenylalanine diet for the first six years of life, or left untreated. Currently, the only certain way of distinguishing between the PKU and hyperphenylalaninemics on the one hand, and hyperphenylalaninemics and PKU heterozygotes on the other, is to measure PAH activity in liver biopsy samples. If the in vivo assay that we have developed can be applied to humans, and there is no apparent reason why it

cannot, liver biopsies will never again have to be used as an aid to diagnosis for PKU and its variants!

The finding that the activity of hepatic PAH can be stimulated in normal rats by administration of synthetic analogues of the pterin cofactor, suggests that it may be possible to raise the levels of PAH in PKU patients to a point where they can be fed a normal diet. It should be emphasized, however, that this possibility depends on whether the mutant form of PAH in PKU patients responds to excess cofactor in the same way as does the normal enzyme.

Proposed Course: Using the in vivo and liver slice assays that we have developed, further attempts will be made to enhance the activity of PAH. The use of the in vivo assay for PAH in humans (normals, parents and siblings of patients with PKU, patients with PKU and patients with hyperphenylalaninemia)will be explored.

Honors and Awards: None

Publications:

Abita, J. -P., Dorche, C. and Kaufman, S.: Further studies on the nature of phenylalanine hydroxylase in brain. Pediat. Res., 1974, in press.

Kaufman, S. and Fisher, D.B.: Pterin-requiring aromatic amino acid hydroxylases. In Molecular Mechanisms of Oxygen Activation (O. Hayaishi, ed.), Academic Press, N.Y., 1974, pp. 285-369.

Serial No. M-NC-2
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biosynthesis of Catecholamines

Previous Serial Number: M-NC-2

Principal Investigator: Seymour Kaufman

Other Investigators: Thomas A. Lloyd, Robert Hoeldtke, Ira Katz

Man Years

Total	:	3.4
Professional	:	2.9
Other	:	0.5

Project Description:

Objectives: These investigations are a continuation of previous studies in this laboratory concerning the biosynthesis and regulation of catecholamines. Since tyrosine hydroxylase has been demonstrated to be the rate limiting step in the biosynthesis of catecholamines, present goals include: characterization of the stimulation of brain tyrosine hydroxylase by phospholipids, isolation and characterization of the endogenous brain tyrosine hydroxylase cofactor, delineation of the biosynthesis of unconjugated pterins in mammalian tissue and studies on the regulation and turnover of mammalian tyrosine hydroxylase under physiological and pharmacological manipulation.

Methods Employed: Bovine caudate tyrosine hydroxylase has been purified 50-fold from an acetone powder extract by relatively standard procedures, yielding a stable preparation which is about 40% pure. The purification of bovine adrenal medulla tyrosine hydroxylase has been extended from a 40% pure preparation to one which is about 90% pure by the addition of anion-exchange chromatography to the previously established purification procedure. Immunochemical studies have been carried out with specific tyrosine hydroxylase-directed antibodies.

Major Findings: 1 - An immunochemical study of the induction of tyrosine hydroxylase in rat adrenal. A wide variety of physiological stresses and drug treatments cause an increase in the synthesis and release of catecholamines in the adrenal medulla. In some of these conditions an increase in the activity of the rate-limiting enzyme, tyrosine hydroxylase, has been observed. In our study, repeated immobilization, cold exposure, and treatment with 6-hydroxydopamine were used to elicit a 2-3 fold rise in tyrosine hydroxylase activity. This increase in activity was observed to be due to an increase in tyrosine hydroxylase-specific protein as measured by immunochemical titrations. The kinetic properties of the induced enzyme were identical to those of the native enzyme. Therefore, our immunochemical titration data indicate that the increase in tyrosine hydroxylase activity after any of the above stresses is due, at least in part, to increased synthesis of normal hydroxylase protein, rather than an "activation" of already existing hydroxylase, or to the induction of a different enzyme species with unique kinetic properties.

2 - The stimulation of partially purified bovine caudate tyrosine hydroxylase by phospholipids. Our interest in the possible interaction of phospholipids with brain tyrosine hydroxylase stemmed from previous studies in this laboratory on the 20-50 fold stimulation of rat liver phenylalanine hydroxylase activity by lysolecithin. Of the phospholipids examined so far, phosphatidyl-L-serine produces the greatest effect. Kinetic analysis has demonstrated that this effect is due to the increase of the affinity of the enzyme for the cofactor, tetrahydropterin, with little or no effect upon the maximal reaction velocity. Since the mucopolysaccharide, heparin, and the poly amino acid, poly-L-glutamate also stimulate brain tyrosine hydroxylase in a similar fashion, the mechanism of this stimulation appears to be of a general polyanionic nature and quite distinct from the phospholipid stimulation of phenylalanine hydroxylase. Although heparin or poly-L-glutamic acid are not likely candidates as naturally-occurring modifiers of brain tyrosine hydroxylase, phosphatidyl-L-serine or other endogenous cerebral phospholipids may play an important role in the regulation of central adrenergic activity.

3 - The apparent double-hydroxylation of phenylalanine by brain tyrosine hydroxylase within synaptosomes. Brain tyrosine hydroxylase is known to hydroxylate phenylalanine nearly as efficiently as it hydroxylates tyrosine when tetrahydrobiopterin serves as the cofactor. Other studies in this

laboratory have shown that when partially purified brain tyrosine hydroxylase hydroxylates phenylalanine twice to dihydroxyphenylalanine, tyrosine is an obligatory free intermediate. In the presence of the endogenous, but unknown, cofactor in rat brain synaptosomes, phenylalanine is also rapidly hydroxylated to tyrosine and dihydroxyphenylalanine. We have recently observed that when ^{14}C-phenylalanine is incubated with synaptosomal preparations, the rate of formation of ^{14}C-dihydroxyphenylalanine is greater than could be accounted for if all of the ^{14}C-phenylalanine was first converted to ^{14}C-tyrosine and equilibrating with the intrasynaptosomal tyrosine pool. This observation could be due to either a) double hydroxylation of phenylalanine on the tyrosine hydroxylase molecule without the release of tyrosine as an intermediate, or b) incomplete mixing of the free ^{14}C-tyrosine intermediate within the synaptosomal tyrosine pool. The possible double-hydroxylation-on-the-enzyme molecule hypothesis has been ruled out by double-labelling experiments, where the hydroxylation of both ^{3}H-phenylalanine and ^{14}C-tyrosine to ^{3}H and ^{14}C-dihydroxyphenylalanine has been measured. We have concluded therefore, that the apparent double-hydroxylation occurs as a result of incomplete pooling of hydroxylase-produced tyrosine with the total tyrosine that is present within the synaptosome.

4 - The subunit structure of pure adrenal dopamine-β-hydroxylase has been determined. The enzyme is composed of 4 subunits, two pairs of which are held together by noncovalent bonds.

It has also been found that the enzyme can be markedly (9-fold) activated by anions such as chloride and bromide. Maximum activation occurs at 0.4 - 0.6 M salt, although activation is detectable at 0.1 M salt.

Scientific Significance: Our finding that the increased activity of tyrosine hydroxylase in response to stress is due to an increased amount of tyrosine hydroxylase protein puts on a firm basis the idea that enzyme induction is actually involved in this response. Now that this point has been established, it should be possible to determine whether the increase in hydroxylase molecules is due to an increased rate of synthesis, a decreased rate of degradation, or both.

The finding that certain phospholipids can stimulate brain tyrosine hydroxylase by a mechanism that involves an increase in the affinity of the enzyme for its pterin cofactor, clarifies a puzzling feature of this enzyme's behavior.

Prior to our observation, there appeared to be a disturbing discrepancy between the concentrations of tetrahydropterin (BH_4) in the brain and the K_m of the hydroxylase for this cofactor, the former value being much smaller than the latter one: there simply did not seem to be enough BH_4 in the brain for it to function as a cofactor for the enzyme. Since phospholipids allow the enzyme to function efficiently with lower amounts of BH_4, this discrepancy has been largely resolved.

The finding that dopamine-β-hydroxylase can be markedly activated by halides uncovers a new way for potentially regulating the activity of this enzyme. Since there is now considerable speculation about a possible defect in this enzyme in brains of schizophrenics, it becomes important to evaluate ways in which the enzyme's activity can be regulated.

Proposed Course: Further studies utilizing the specific tyrosine-hydroxylase-directed antiserum should allow determination of the turnover time of this critical biosynthetic enzyme under a variety of conditions. Additional studies are planned to determine whether an increase in protein synthesis or a decrease in protein degradation account for the increased amount of protein observed after induction of tyrosine hydroxylase by physiological or pharmacological manipulation.

Current procedures for purifying bovine caudate tyrosine hydroxylase have yielded a preparation sufficiently pure to use for a thorough characterization of this enzyme. The rate of endogenous phospholipids or other polyanions in regulating the activity of this enzyme is under current investigation.

Honors and Awards: None

Publications:

Hoeldtke, R., Lloyd, T. and Kaufman, S.: An immunochemical study of the induction of tyrosine hydroxylase in rat adrenal glands. Biochem. Biophys. Res. Commun., 1974, in press.

Craine, J. E., Daniels, G. H. and Kaufman, S.: Dopamine-β-hydroxylase. The subunit structure and anion activation of the bovine adrenal enzyme. J. Biol. Chem. 248: 7838-7844, 1973.

Kaufman, S.: Cofactors of tyrosine hydroxylase. In Frontiers in Catecholamine Research (E. Usdin & S. Snyder, eds.), Pergamon Press, N.Y., 1973, pp. 53-60.

Serial No. M-NC-4
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Biochemical Basis of Skeletal Muscle Hypertrophy

Previous Serial Number: M-NC-4

Principal Investigator: Seymour Kaufman

Other Investigator: Richard Bailyn

Man Years:

Total	:	1.6
Professional	:	1.3
Other	:	0.3

Project Description:

Objectives: The objective of this project remains the elucidation of the biochemical correlates of skeletal muscle hypertrophy.

Methods Employed: A tenotomy operation as described previously is employed to produce work-induced skeletal muscle hypertrophy in the rat. Biochemical fractions of interest are labelled with radioisotopes. Nucleic acids are recovered using a modified Schmidt-Tannhauser procedure. Collagen is measured by the Prockop-Udenfriend method. Phospholipids are separated according to the Folch procedure.

Major Findings: Previous work in this laboratory has demonstrated that work-induced skeletal muscle cell hypertrophy is accompanied by connective tissue cell proliferation and that the enhancement of RNA synthesis associated with muscle hypertrophy is localized to these proliferating cellular elements. The possibility that connective tissue cell proliferation is a necessary step in muscle hypertrophy was examined. Through the use of the drugs, hydroxyurea and cytosine arabinoside, DNA synthesis and cellular mitotic acitivity are almost completely inhibited without significantly affecting the development of muscle hypertrophy.

Scientific Significance: The above studies demonstrate that connective tissue proliferation is not a necessary step in the development of muscle hypertrophy. These studies also shed doubt on the hypothesis that collagen or other biosynthetic products of connective tissue cells are responsible for the initiation of muscle hypertrophy.

Proposed Course: Experiments will be established in hopes of confirming the suspicion that collagen synthesis does not occur in a hypertrophying muscle in which DNA synthesis and cellular mitoses are inhibited. This will definitively exclude any role for collagen synthesis in the early stages of muscle hypertrophy.

We have previously demonstrated that muscle hypertrophy is also associated with the stimulation of phospholipid metabolism. The question of whether this phenomenon is localized to the hypertrophying muscle cell (and thus of primary importance) or to the proliferating connective tissue cell (and thus of only secondary interest) is to be answered using autoradiographic techniques.

Honors and Awards: None

Publications:

Jablecki, C.K., Heuser, J.E., and Kaufman, S.: Autoradiographic localization of new RNA synthesis in hypertrophying skeletal muscle. J. Cell Biol. 57, 743-59, 1973.

Serial No. M-NC-5
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Process of Lysogeny

Previous Serial Number: M-NC-5

Principal Investigator: Howard A. Nash

Man Years

Total	:	2
Professional	:	1
Other	:	1

Project Description:

Objectives: The project is a continuing study of the processes involved in the insertion of viral DNA into the host genome. An elucidation, at the level of biochemistry and molecular biology, of the steps involved in DNA-DNA recombination is the overall goal.

Methods Employed: Microbiological techniques for isolation, growth, genetic characterization of bacteriophages; radioactive and/or density labeling procedures for bacteriophage; gradients in a high speed preparative ultracentrifuge; polyacrylamide gel electrophoresis; chromatographic column separations.

Major Findings: The *in vitro* analysis of recombinant DNA first described in this laboratory has been used to investigate the relationship between gene expression and site-specific integrative recombination. This recombination, normally used to insert the genome of a virus into that of its host, has been shown to interact in a complicated way with other macromolecular transformations. On the one hand, the fundamental reactions involved in integrative recombination - aligning, cutting, switching and rejoining two regions of double helix - have been shown to be independent of DNA replication and/or transcription. On the other hand, transcriptional expression has been shown to modulate the effectiveness of integrative recombination in at least two ways. First, transcriptional expression of a molecule renders it a poor candidate for recombination. Interference with this

expression by immune repressor or by chemical inhibitors augments the appearance of recombinant DNA. This effect of transcription may be related to changes in subcellular localization of the phage genome rather than creation of a transcript. Second, host transcriptional expression is required for integrative recombination in the presence of a phage gene product normally involved in a different site-specific recombination. A host dependent rearrangement of phage coded proteins appears to be implicated in this phenomenon.

The characteristics of integrative recombination have been investigated in detail in cells treated with inhibitors of RNA and protein synthesis. Recombinant DNA accumulates linearly with time for 15-20 minutes after infection of suitably primed cells. The recombination enzymes are stable at 31° but are rapidly and irreversibly inactivated at temperatures above 41°C. Integrative recombination is blocked in the presence of the metabolic inhibitors cyanide or dinitrophenol, indicating a dependence on high energy components in the cell.

An *in vitro* system for the creation of site-specific recombinant DNA has been developed. The system utilizes the same primed hosts and substrate phage used in earlier work on integrative recombination in whole cells. The recombinant DNA is assayed by the *in vitro* transfection analysis developed in this laboratory. The system has been shown to reflect normal integrative recombination in its dependence on *int* gene product, limitation to recombination of specialized attachment sites, inactivation by temperatures over 41°C, and dependence on high energy phosphate compounds.

Scientific Significance: Recombination is one of the fundamental transformations of genetic information. It is involved in the maintenance of genetic integration, the creation of genetic variability, and in the physical relationship between self and foreign DNA. This work represents the first investigation of the biochemistry of recombination *in vitro*. First, we have developed a reliable method of analysis for recombinant DNA. Second, we have used this method to define optimum conditions for production and utilization of recombination enzymes *in vivo*. Finally, this information has been used to develop a system for *in vitro* production of recombinant DNA. Previous work has been limited to the study of enzymatic activities associated with genetic elements involved in recombination; many important biochemical elements have been unavailable for study. The development of an *in vitro* system that performs genetic recombination at high efficiency opens this field to logical and orderly investigation.

Proposed Course: The in vitro formation of recombinant DNA will be exploited in several ways. The enzymatic components of the reaction will be identified and purified, the cofactor requirements will be analyzed and intermediates in recombination will be searched for. Extension of the methods utilized for analysis of other recombination systems will be initiated.

Honors and Awards: None

Publications:

Nash, H. A.: λattB-attP, A λ derivative containing both sites involved in integrative recombination. Virology 57: 207-216, 1974

Nash, H. A.: Purification of bacteriophage λ int protein. Nature 247: 543-545, 1974.

Serial No. M-NC-6
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Physicochemical Investigations of Biofunctional Structures of Glycosaminoglycans, Glycolipids and Complex Polysaccharides; Polysaccharide-Polypeptide Interactions

Previous Serial Number: M-NC-6

Principal Investigator: Audrey Larack Stone

Man Years

Total	:	0.3
Professional	:	0.3
Other	:	0

Project Description:

Physicochemical measurement of optical conformations of complex polysaccharides and the glycosaminoglycans (GAG, mucopolysaccharides); determination of the molecular basis for the formation and maintenance of inter- and intra-molecular structures known to be involved in the rapid transformation of simple cations (and biogenic amines) from the bound to the active form; and the conformational constraints induced in basic polypeptides as a consequence of interactions with anionic polysaccharides.

Objectives: To elucidate the particulars of the various molecular conformations of GAG and of their complexes with polypeptides and biogenic amines by means of their ultraviolet amide transition bands (M-NC-21, 1967) and/or the special optical effects of their dye complexes (M-NC-21, 1965) with the view towards understanding the molecular basis for biological activities associated with interstitial and intracellular matrices. To demonstrate molecular conformations in solution of variously related complex polysaccharides (known to form helical structures in oriented fibers), using techniques developed earlier in this project. To determine specificity if any, in the conformation - directing effect of anionic polysaccharides on polypeptide structures.

Major Findings: I - Standard GAG exhibit particular CD in the spectral region of the n-π and π-π amide transitions (see M-NC-6, 1971). Metachromatic reactions of methylene blue when bound to various acidic polysaccharides show

specific properties dependent upon the structure of the polymer (see M-NC-6, 1970). The latter technique demonstrated that kappa-Carrageenate assumes a net, right-handed helical conformation in solution while the closely related lambda-Carrageenate chain from the same organism assumes an opposite handedness in solution (see M-NC-6, 1973). The conformation-directing effect (first described by Gelman, R. A., et al.) of purified acidic polysaccharides (PS) from plant, bacterial and animal sources on poly-L-lysine (PLL) at neutral pH were studied showing that although charge density was an important factor in the degree to which these PS promote α-helical conformation, there was little specificity in that reaction in regard to the primary structure or secondary and tertiary conformations of the PS. Thus, polysulfates, polyuronates, and mixed polyanionic groups on a homo or complex polysaccharide backbone, all could promote α-helical order in PLL, provided the anionic density was sufficient.

A difference in the nature of the conformation-directing effect (i.e., α-helix, β-structure, extended helix, etc.) was seen among the carrageenans, which have different conformations in solution (see above) and in X-ray studies. Kappa and iota carrageenan are strong α-helix promoting, while interactions with lambda yield mixed PLL conformation including some β-structures.

II - Preliminary studies on the optical properties of dihydropteridine reductase showed that the enzyme has a high α-helix content. Studies on the optical properties of biogenic amines in regard to putative specific complexes with anionic polysaccharides show that the CD of L-norepinephrine has two negative bands (about 2205 Å and 2035 Å). In the presence of the highly anionic polysaccharide, heparin, the CD of L-norepinephrine is altered.

Scientific Significance: I - These studies help to separate and evaluate the importance of various aspects of PS structure and charge in the in vitro model for interactions between polysaccharides and changing protein structures. The finding that lambda carrageenate can alter PLL structure to form β-protein structures is particularly important because it provides a molecular basis for forming insoluble, intracellular membranes from soluble protein. Thus, enzymes (such as those involved in catecholamine biosynthesis) may become membrane-bound by a process in which neighboring proteins become insoluble due to the β-conformation-directing effect of a

specific polyanion. It is of interest that most of the conformation-directing effects seen in vitro with PLL are towards the α-helix.

II - Further approaches to the study of in vitro complexes of the biogenic amines are just beginning. The preliminary results indicate that the optical methods developed previously can be extended to explore molecular mechanisms for catechol amine storage and release.

Proposed Course: I - Investigation of the β-conformation directing effect of lambda carrageenan on PLL will be extended to a study of the birefringeance of congo red stained preparations and to X-ray analysis of fibrous precipitates of their complexes. Interaction of L-arternerol with other polyanions and quantitative analysis of the optical properties will be undertaken to determine whether these substances might sequester catechol amines because of strong interactions based upon non-polar as well as electrostatic interactions.

Honors and Awards:

Referee for Biopolymers
Referee for Journal of Molecular Biology
Referee for Analytical Biochemistry
Referee for Thrombosis Research

Invited by Dr. Roy Whistler, editor of Methods in Carbohydrate Chemistry, to contribute an article entitled "Optical Rotary Dispersion and Circular Dichroism in Polysaccharide Structural Analysis" for Volume VII (1974).

Publications:

Stone, Audrey L.: Polysaccharide-polypeptide interactions. VII International Symposium on Carbohydrate Chemistry, Bratislava, 1974, in press.

Serial No. M-NC-7
1. Neurochemistry
2. Biophysical Chemistry
3. Bethesda, Md.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Role of the Cell Membrane in Cellular Organization, A Molecular Study

Previous Serial Number: M-NC-7

Principal Investigator: David M. Neville, Jr.

Other Investigators: Hartmut Glossmann, Ta Min Chang; Ronald Kahn, Andrew Soll, Pierre DeMeyts and Karla Megyesi, NIAMD

Man Years

Total	4.0
Professional	3.0
Other	1

Project Description:

Objectives: The aim of this project is to determine the specific chemical interactions which occur at the surfaces of cells which affect cellular differentiation and cellular organization. Specifically we propose that the recognition which cells have of their solid environment and neighboring cells is mediated by specific proteins on the surface membranes. In addition, the surface of each cell has protein or glycoprotein receptors for a variety of soluble hormones and transmitter substances. Our aim is to study the interaction between the receptor molecules and the soluble and fixed transmitter molecules so that the interaction which leads to altered cell function can be quantitatively defined. Having done this we propose to examine these interactions in pathological states in order to determine if altered receptors or receptor concentration play a role in disease states.

Methods Employed: Ultracentrifugation, disc electrophoresis, gel filtration, immuno-diffusion, fluorescence microscopy, circular dichroism, tissue and organ culture, radio receptor assays.

Major Findings: 1. The surface membrane insulin receptor population, which determines to a large extent an animals'

insulin sensitivity or resistance, has been shown to be under a negative feedback control from free insulin. This has been demonstrated in tissue cultural cells where competing variables could be controlled. Therefore, the decreased insulin receptor population seen in the insulin resistant states of obesity and hyperglucocorticoidism appears secondary to hyperinsulinemia.

2. The surface membrane insulin receptor population exhibit negative cooperative interactions. Increased occupancy of sites increases the rate of dissociation of insulin from occupied sites. The average affinity of a cell for the hormone is dependent not only on the number of sites but the extent of the cooperative interactions.

3. In tissue culture, serum and high concentrations of insulin are both known to have growth stimulating properties. Liver cell surface membranes have been shown to possess a receptor for a peptide present in serum known as NSILA-s (non-supressible insulin like activity - ethanol soluble). Insulin also binds to this receptor but is less active by a factor of 300,000. NSILA-s has insulin like activity *in vivo* and displaces insulin from its receptor in proportion to its insulin like activity (1/300 of insulin on a weight basis).

Scientific Significance: 1. The demonstration of receptor population feedback control *in vitro* enables investigations into the biochemical mechanism of receptor control. Since receptors are altered in some disease states it may be possible to correct the pathology by altering the receptor control mechanisms with pharmocologic agents.

2. The demonstration of a distinct NSILA-s receptor for a circulating peptide in the nanomolar concentration range indicates that there are probably a wide variety of unknown peptides which act specifically on certain cells via specific receptors to alter cell function. NSILA-s action is probably growth promoting. It is interesting to note that hypoglycemia occurs in certain cases of human malignancy which is not due to hyperinsulinemia, but could be explained by the weak binding of NSILA-s to the insulin receptor.

Honors and Awards:

Commissioned Corps Commendation Medal, January, 1974

Publications:

Kahn, C. R., Freychet, P., Neville, D. M., Jr., and Roth, J.: Quantitative aspects of the insulin-receptor interaction in liver plasma membrane. J. Biol. Chem. 249: 2249-2257, 1974.

Gavin, J. R. III, Roth, J., Neville, D. M., Jr., DeMeyts, P., and Buell, D. N.: Insulin-dependent regulation of insulin receptor concentrations: A direct demonstration in cell culture. Proc. Nat. Acad. Sci. 71: 84-88, 1974.

Megyesi, K., Kahn, C. R., Roth, J., Froesch, E. R., Humbel, R. E., Zapf, J., and Neville, D. M., Jr.: Insulin and non-suppressible insulin-like activity (INSILA-s); evidence for separate plasma membrane receptor sites. Biochem. Biophys. Res. Commun. 57: 307-315, 1974.

Soll, A. H., Goldfine, I. D., Roth, J., Kahn, C. R., and Neville, D. M., Jr.: Thymic lymphocytes in obese (ob/ob) mice: A mirror of the insulin receptor defect in liver and fat. J. Biol. Chem., in press.

Kahn, C. R., Soll, A. H., Neville, D. M., Jr., Goldfine, I. D., Archer, J. A., Gorden, P., and Roth, J.: The insulin receptor in obesity and other states of altered insulin sensitivity. Proceedings of the Fogarty Conference on Obesity, Oct. 1-3, 1973, Bethesda, Md.

DeMeyts, P., Roth, J., Neville, D. M., Jr., Gavin, J. R. III, and Lesniak, M. A.: Insulin interactions with the receptors: Experimental evidence for negative cooperativity. Biochem. Biophys. Res. Commun. 55: 154-161, 1973.

Goldfine, I. D., Kahn, C. R., Neville, D. M., Jr., Roth, J., Garrison, M. M., and Bates, R. W.: Decreased binding of insulin to its receptors in rats with hormone induced insulin resistance. Biochem. Biophys. Res. Commun. 53: 852-857, 1973.

Freychet, P., Roth, J., Kahn, R., and Neville, D. M., Jr.: Interaction de l'insuline avec ses receptors hepatiques: Liaison specifique de l'insuline radio-iodee aux membranes plasmiques. Colloque sur les hormones pancreatiques, November, 1972.

Glossmann, H., and Neville, D. M., Jr.: Phlorizin receptors in isolated kidney brush border membranes. Differential enzymatic modification of high-affinity receptors and unspecific binding sites. Biochim. Biophys. Acta 323: 408-414, 1973.

Neville, D. M., Jr.: Isolation of cell surface membrane fractions from mammalian cells and organs. In Methods in Membrane Biology, vol. 3, E. Korn, ed., Plenum Publishing Corporation, in press.

Neville, D. M., Jr.: Receptors for polypeptide hormones: Direct studies of insulin binding to purified liver plasma membranes. Submitted to In Vitro, August, 1973.

Serial No. M-NC-8
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biological and Biochemical Models for the Genetic Disease, Phenylketonuria (PKU); Subproject 1: Development of a Diagnostic Assay for PKU in Tissue Culture; Subproject 2: Chemically Induced Hyperphenylalaninemia

Previous Serial Number: M-NC-8

Principal Investigator: Audrey L. Stone

Other Investigators: Seymour Kaufman, William C. Crafford; Subproject 1 - Elizabeth Neufeld, LBC, NIAMDD; Subproject 2 - Seymour Kaufman, Norman Chang

Man Years

Total	2.3
Professional:	1.3
Other :	1.0

Project Description:

Development of a murine model for PKU using X-irradiated sires and a screening test for serum phenylalanine and tyrosine levels as a guide for heterozygosity for the phenylalanine hydroxylase gene in their offspring with normal females; investigation of natural history of the enzyme with cell models, using murine human and primate organs; search for mutants defective in the cofactor enzyme, dihydropteridine reductase (DPR). Subproject 1: Development of human cell models for PKU studies using skin fibroblast and amnionic cell lines. Investigation of experimental lymphoid tissue and cells. Subproject 2: To induce hyperphenylalaninemia in neonatal rats.

Objectives: 1) To produce and develop a mutant, murine strain with the genetic defect of PKU; to examine organs and cells of human, primate and other species for the expression of phenylalanine hydroxylase (PAH) activity; 2) to compare in particular the biological and biochemical properties of phenylalanine hydroxylase from human liver and kidney cells

and other tissues; 3) to examine cofactor reductase as another possible mode of PAH deficiency. Elucidation of the biological range and function of dihydropteridine reductase in normal tissues and cell lines and in deficient mutants.

Subproject 1: Development of a prenatal diagnostic test for PKU homozygosity using amnionic cell lines and/or a postnatal diagnostic test for PKU using skin fibroblasts or lymphoid cell lines.

Subproject 2: To analyze the fidelity to clinical PKU of chemically-induced hyperphenylalaninemia in animals.

Methods: See M-NC-8, 1971, 1972 and 1973. Dihydropteridine reductase (DPR) was assayed by the spectrophotometric method of Craine and Kaufman. Chronic inhibition of DPR, in vivo in the mouse, was elicited and studied using intraperitoneal administration of 2 mg/kgm of methotrexate in saline solution for 3 and 5 consecutive days.

Subproject 2: Thrice-daily injections of high doses of L-phenylalanine have been injected into neonatal rats from 5 days after birth to 2 to 3 weeks after birth. Myelin in the brain was then isolated and analyzed.

Major Findings: This project has moved forward since the last report yielding, in particular, essentially new information concerning the interrelation of regulation of phenylalanine hydroxylase and its cofactor reductase (DPR) in vivo - both in the suspect mutants and in experimentally-developed DPR insufficiency. In M-NC-8 (1973) it was reported that four offspring of X-irradiated sires had 1/2-2/3 the level of hepatic DPR. The unexpected correlation with relatively high serum phenylalanine to tyrosine ratio, ($^{P}/_{T}$) (indicating some impairment of the phenylalanine hydroxylating system) was noted and signified that DPR levels may influence the expression of phenylalanine hydroxylase. Since then, the suspect animals (infertile and old) were sacrificed. Their livers again exhibited 50-60 percent DPR levels and the serum $^{P}/_{T}$ was high. In addition direct measurement of PAH levels showed these to be reduced to 50-60 percent of normal. Furthermore, the DPR of other organs, such as the brain and adrenals was also lower than normal.

In experimentally-developed DPR insufficiency, the same correlation between reduced DPR levels and PAH activity in the liver was observed, i.e., after 5 days of methotrexate treatment the DPR of the liver was 50-60 percent of normal

and the level of PAH in the liver fell to ~30 percent of normal. These results will be reported in a manuscript in progress entitled "The Effect of Experimentally-Induced Dihydropteridine Reductase Insufficiency on the Phenylalanine Hydroxylating System in vivo". Possible explanations on the effects will be discussed therein.

Due to the generosity of LGCB which lends us 3 rows on one animal rack in an animal room for mating animals, we have been regularly producing "suspect offspring" (~25/mo) since 12/73. Using the findings (M-NC-8, 1973) that (a) 4 of 60 mice screened by partial hepatectomy showed significantly reduced levels of DPR and (b) this was accompanied by relatively high $^{P}/T$ ratios (although in the range considered normal for the PKU screening), a monitoring of putative "DPR" heterozygotes on the basis of $^{P}/T$ supports that this abnormality occurs more frequently than that of the high $^{P}/T$ associated with PKU.

Preliminary investigation of parameters of PAH and DPR activities in the kidney showed that most (75 percent) of the PAH activity was found in the medulla while 50 percent of the DPR was in the cortex which also had the highest specific activity. The specific activity in the medulla was ~80 percent of the cortex.

Major Findings Subproject 1: It was reported last year that skin fibroblasts (human tissue cultures) assayed for PAH activity exhibited low levels of radioactive product cochromatographing with tyrosine. The level was below comfortable limits for the distinction between the normal and suspect PKU infant and therefore failed to provide the desired clinical application. Several new attempts were made to alter the expression of the PAH gene in cell cultures. These failed to yield higher values. Results showed that there was no specific inhibitor present in the cell lysates, however. Progress in these studies has been frustrated not only by the fact that the obvious possibilities of increasing PAH detection or expression have failed, indicating that new approaches need to be explored, but also by the need for a post-doctorate-level support person to regularly conduct new experimental leads rather than the setting aside by the principle investigator of a week, nights, weekends from the regular project schedules when possible.

Major Findings Subproject 2: In confirmation of published reports, a defect in myelin formation can be induced in the brains of neontal rats by the injection of high doses of

phenylalanine. Since there is evidence that indicates that there is less myelin in the brains of PKU patients, by this criterion the chemical model for PKU appears to be an adequate one.

Scientific Significance: The mini-scale screening program for suspect PKU (see M-NC-8, 1971 and 1972) heterozygotes continues. The continued studies of DPR insufficiency (see M-NC-8, 1973) have shown that a decrease in the level of liver DPR to two thrids or less is reflected not only in plasma P/T but also intercepts the expression PAH activity causing a concommitant decrease. How this "regulatory" interaction operates is still a new question, but is subject to at least two distinct kinds of explanation, i.e., an alteration of biochemically determined reactions or an alteration of reactions determining genetic expression. These explanations are discussed in the above manuscript. If phenylalanine metabolism is important in the early development of the new-born human brain (as is indicated by the PKU-ologists) then an understanding of the new regulatory possibility reported herein is an obligate undertaking. Furthermore, the DPR insufficiency can be elicited experimentally in adult mice. What are the consequent subtle changes in brain function and biogenic amine functions?

Scientific Significance Subproject 1: The value of a safe diagnostic test for PKU is evident. The failure to find a suitable cell model using the current methods and thinking leads to the conclusion that basic changes must be induced in these cells to foster the expression of the PAH capability.

Scientific Significance Subproject 2: Now that the chemical model for PKU has been shown to share with the human disease a characteristic lesion, it should be possible to explore specific mechanisms for the pathophysiology of PKU. Perhaps the most popular theory for how high phenylalanine leads to mental retardation assumes that high phenylalanine interferes with the transport into the brain of a whole family of amino acids. A logical extension of this idea is that excessive amounts of any member of this family of amino acids should lead to the same defect in myelin synthesis as that caused by high phenylalanine concentrations.

Proposed Course: The screening program will be continued as planned, as will be the basic studies on the correlation between DPR and PAH expression. In this latter regard it is important to measure the actual amounts of protein enzyme present in the organs as well as the expression of their activities *in vivo* and *in vitro*. The subproject 1 will be

followed when support persons are available. Dr. Uhlendorf is interested in providing the amnionic cells. If it were possible to grow up certain lines of these in adequate quantity they may exhibit PAH activity without special manipulation.

Proposed Course Subproject 2: Other members of the family of amino acids that are believed to share with phenylalanine a common transport system into the brain will be studied to see if they can also induce a defect in myelin synthesis.

Honors and Awards: None

Publications: None

Serial No. M-NC-9
1. Neurochemistry
2.
3. Bethesda, Md.

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Conversion of Tryptophan to 5-Hydroxy-tryptophan

Previous Serial Number: M-NC-9

Principal Investigator: Seymour Kaufman

Other Investigators: Jeffrey Tong

Man Years

Total	:	1.2
Professional	:	1.2
Other	:	0

Project Description:

Objectives: The conversion of tryptophan to 5-hydroxy-tryptophan is believed to be the rate-limiting step in the biosynthesis of the neurotransmitter, serotonin. The reaction is catalyzed by the mixed-function oxidase, tryptophan hydroxylase. The goal of this project is to isolate the enzyme in order to study its regulatory properties.

Methods Employed: Standard methods of protein purification, including salt fractionation, ion-exchange chromatography, and sephadex-gel filtration, have been used.

Major Findings: During the attempts to purify tryptophan hydroxylase from rabbit hindbrains, it was found that whenever an ammonium sulphate fractionation was performed the enzyme could no longer be separated from the bulk protein by classical purification methods, e.g., gel filtration or ion exchange chromatography. The use of detergents or chaotropic ions at concentrations tolerated by the enzyme did not overcome the "aggregation phenomenon". With this knowledge, the use of high concentrations of salt in the purification procedure was avoided and tryptophan hydroxylase has now been extensively purified. Disc electrophoresis of the purified enzyme showed one major protein band which matches with enzyme activity.

Even though tryptophan hydroxylase is believed to be the rate limiting enzyme in the biosynthesis of the putative neurotransmitter 5-hydroxytryptamine (serotonin), relatively little is known about its regulatory mechanisms. A possible mode of regulation is by enzyme-catalyzed phosphorylation and dephosphorylation sequences. A time-dependent inactivation of tryptophan hydroxylase occurs in the presence of ATP and Mg^{2+} *in vitro*. Both the rate and extent of the inactivation showed a marked dependence on temperature, and were independent of adenosine 3',5'-monophosphate from 10^{-3} to 10^{-6} M. Although the evidence is consistent with a mechanism that tryptophan hydroxylase is regulated by phosphorylation and dephosphorylation of the enzyme, it has not yet been possible to demonstrate the latter reaction.

Another interesting finding on the possible regulatory mechanism is that treatment of crude or partially purified rabbit hindbrain tryptophan hydroxylase with limited tryptic or chymotryptic digestion results in a two-fold increase in enzyme activity, when the enzyme is assayed with 2-amino-4-hydroxy-6-methyltetrahydropteridine as cofactor. This observation is reminiscent of the proteolytic activation of phenylalanine and tyrosine hydroxylases

Scientific Significance: The procedure for the purification of brain tryptophan hydroxylase that we have developed will allow us, for the first time, to characterize the enzyme in terms of its structure. It will also facilitate the preparation of a specific antiserum to the enzyme. This antiserum should be a valuable tool in the mapping of serotonergic pathways in the central nervous system.

Proposed Course: Further purification to the point where a pure enzyme is available. Structural and immunological studies on the pure enzyme will then be undertaken.

Honors and Awards: None

Publications: None

Serial No. M-NP-3
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Muscle function and metabolism

Previous Serial Number: Same

Principal Investigators: S. I. Rapoport and J. Vergara

Other Investigators: J. Bidinger, J. V. Passonneau

Cooperating Units: Laboratory of Neuropathology
National Institute of Neurological
Diseases and Stroke

Man Years:

Total:	2.24
Professional:	1.50
Other:	.74

Project Description:

Objectives: To study the relations among ionic regulation, electrical properties, contraction and metabolism in muscle. In particular, to study the mechanisms of fatigue and of the control of muscle contractility.

Methods Employed: (a) Twitch and tetanic tensions are recorded from muscles with transducers, amplifiers and pen recorders. Muscles are stimulated periodically with standard neurophysiological techniques. Ionic determinations of Ca, Mg, Na and K are made after measuring wet and dry weights, ashing in a furnace, and using emission and atomic absorption spectrophotometry. Data are analyzed with computer techniques.

(b) Single muscle fibers of frog muscles are isolated and set up in a temperature-controlled flow chamber. They are stimulated under different conditions of temperature, solution concentration, stretch and stimulus parameters. Oscilloscope tracings of twitch and its time derivatives are photographed and taped. Records are analyzed by computer techniques.

Major Findings: (a) Muscles in a Ringer bath at room temperature lose their ability to contract in response to either a single stimulus or to a tetanic (repetitive) stimulus. They slowly accumulate sodium and lose their normal potassium. The whole process takes about 48 hours. When sartorius muscles of frog are stretched by 20-30%, the loss of contractility, and the changes in sodium and potassium contents, are retarded. Stretched muscles have more potassium and less sodium at 48 hours than do unstretched muscles. We suggest that stretch retards the running down of ionic homeostasis by stimulating the sodium-potassium exchange pump, either directly or through a general stimulation of metabolism. The retardation of loss of contractility is probably mediated by the effect on ionic homeostasis. The Na-K pump actively transports potassium into muscle and sodium out of muscle. It has been characterized theoretically by me (Rapoport, S.I.: Biophysical Journal, 10, 246-259, 1970: Biophysical Journal, 11, 631-647, 1971). Inhibition of the pump by ouabain, cold or potassium-free Ringer abolishes differences between stretched and unstretched muscles in ionic contents and rate of loss of contractility.

(b) Single muscle fibers of the frog semitendinosus were dissected and stimulated extracellularly in a controlled flow bath. Tetanic stimulation (20 shocks/sec for 50 seconds) reduced the twitch tension to 60% of its initial value. This reduction is defined as "fatigue" of twitch tension. Tension returned to its initial level in 80 to 200 minutes.

Tetanic stimulation of single fibers in hypertonic solutions (made by adding NaCl or sucrose to the bath) did not produce a posttetanic reduction in twitch tension (measured after the muscles were returned to isotonic Ringer). Hypertonic solutions block contraction but not the excitation of muscle nor the release of calcium. Therefore, these experiments demonstrate that fatigue of twitch tension is due to effects at the contraction stage of the excitation-contraction process in muscle. Previous reports localized fatigue elsewhere (e.g. Eberstein & Sandow, 1963). An abstract of this work has been published: Vergara, J. and S.I. Rapoport. Fatigue in the process of excitation-contraction of frog muscle, as studied with hypertonic solutions. J. General Physiology., 62, 656, 1973.

We thought that fatigue of twitch was due to metabolic exhaustion, probably because ATP levels in muscle were reduced. This suggestion is supported by our observation that single fibers that are stimulated in glucose-Ringer do not fatigue, and that in fact ATP levels in fatigued muscles are reduced (work done in collaboration with J. Passoneau).

Significance to Bio-medical Research and Program of the Institute: The relations in muscle among metabolism, regulation of ionic homeostasis and contraction are not understood clearly. The interaction among these fundamental mechanisms are important to the comprehension of basic muscle physiology and the more complicated analysis of muscle disease.

We have shown that (a) mechanical properties of muscle (stretch) regulate contractility and ionic contents, probably by stimulating metabolism and the sodium-potassium exchange pump, and (b) metabolism in turn regulates twitch and tetanus in single fibers. Fatigue is evident at the level of contraction of the excitation-contraction process in muscle. It is now possible to construct a more meaningful feedback relationship between metabolism, ionic content and muscle contraction.

Proposed Course of Project: We shall continue the study of twitch and the fatigue process in single muscle fibers, and try to correlate quantitatively metabolic changes with the state of fatigue. The work on stretch in whole muscle is completed.

Honors and Awards:

Invited Participant: Fogarty International Center, Bethesda, April, 1974. Symposium on Muscle Contraction.

Publications:

Rapoport, S.I. and J.M. Bidinger. The effect of stretch on contractility and ionic contents of the sartorius muscle of Rana pipiens. American Journal of Physiology, 226: 452-457, 1974.

Serial No. M-NP-8
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Transport Mechanisms Across Membranes and The Blood-Brain Barrier

Previous Serial Number: Same

Principal Investigators: S. I. Rapoport

Other Investigators: H. Thompson, K. Matthews, M. W. Brightman, T. S. Reese, H. Levitan, T. Kuwabara

Cooperating Units:
(a) Laboratory of Neuropathology and Neuroanatomical Sciences, National Institute of Neurological Diseases and Stroke

(b) Department of Zoology, University of Maryland, College Park, Maryland

(c) Laboratory of Vision Research, National Eye Institute

Man Years:

Total:	2.26
Professional:	1.25
Other:	1.01

Project Description:

Objectives: To understand the nature and function of the blood-brain barrier, and to amplify the theory of active and passive transport across cellular membranes.

Methods Employed: Surgical procedures are used for opening the skull and dura of rabbit and monkey, isolating and perfusing the internal carotid artery. Neurological examinations are performed and electroencephalograms used to determine brain function, and methods of perfusion and fixation for brain and eye histology are applied. Neurophysiological techniques are used to record DC and pH potentials on the surface of the cerebral cortex. Mathematics and the theory of irreversible thermodynamics are used to consider results quantitatively and to formulate and test a model

for the osmotic action of substances on the blood-brain barrier. Computers are used for data analysis and model formulation. Electron microscopy is used to determine tracer passage and subcellular morphology.

Major Findings: (a) Theory and physiological and morphological demonstration of osmotic opening of the blood-brain barrier: The following work was done in collaboration with M.W. Brightman and T.S. Reese. The blood-brain barrier in the mammal can be osmotically opened in a reversible manner by shrinking cerebrovascular endothelial cells and separating the tight junctions that connect them. The tight junctions make the vascular endothelium act like a continuous cell layer which restricts exchange of large water soluble solutes and electrolytes between blood and brain. The application of concentrated solutions of salts, urea, lactamide and other slightly lipid soluble non-electrolytes to the surface of the brain in rabbits opens up the barrier reversibly at the pia-arachnoid vessels. Injection of these substances into the internal carotid artery leads to reversible opening of the cerebrovascular endothelium over the whole cerebral cortex of one hemisphere. The theory of osmotic opening, which I had proposed on the basis of previous experiments, (Rapoport, S.I., Amer. J. Physiol., 219, 270, 1970 and Rapoport, S.I., Intern. J. Neurosci., 2, 1, 1971) was extended and confirmed by electron microscopy, using peroxidase as an electron-dense tracer.

(b) Effect of osmotic opening of the blood-brain barrier on long term survival and neurological function in the monkey. This work is being done in collaboration with H.K. Thompson. When we used the usual method in the monkey for perfusing hypertonic solutions into the brain (Steinwall, Acta Radiol., 49, 281, 1958), we found that when the blood-brain barrier opened there was associated neuronal damage with clinical signs of motor weakness in the animals just recovered. This might imply that opening the blood-brain barrier itself leads to brain damage in chronic experiments, and would restrict the eventual use of osmotic opening as a pharmacological tool. We guessed that the permanent ligation of the common carotid artery in the Steinwall procedure caused the neuronal damage, and not osmotic opening itself. We therefore developed a new perfusion method, perfusing solutions through the lingual artery when clamping temporarily the external and common carotids. The results changed dramatically, and about 90% of the monkeys with osmotic barrier opening had no obvious neurological damage.

(c) Equi-osmolal opening of the blood-brain barrier by X-ray contrast media used in carotid arteriography. This work was done in collaboration with J. Bidinger and H. K.

Thompson. Each of six X-ray contrast media of differing composition were perfused into the internal carotid artery of rabbits for 20 seconds. Each opened the blood-brain barrier at mean osmotic threshold of 1.2 osmolal, not different from the threshold osmolality for NaCl. The approximately equi-osmolal threshold for barrier opening by the X-ray contrast media and by NaCl suggests that the contrast media osmotically open the tight junctions between cerebrovascular endothelial cells, probably by osmotically shrinking the cells themselves.

(d) Dependence of neurotoxicity of X-ray contrast media on lipid solubility. This work is theoretical and is being done in collaboration with H. Levitan. When I recognized that the X-ray contrast media opened up the barrier osmotically at equi-osmolal concentrations, it became obvious that a factor other than osmotic barrier opening determined neurotoxicity. Neurotoxicity can differ markedly from one medium to another. We rank ordered clinical neurotoxicity of 8 X-ray contrast media and calculated their olive oil/water partition coefficients, which are classical parameters of lipid solubility. The correlation between lipid solubility and neurotoxicity was significant at the $P<0.05$ level. We proposed that the more lipid soluble the agent, the faster it enters the brain across the blood-brain barrier. We are now able to design series of X-ray contrast agents which are less lipid soluble than any in current use. We hope that they can be synthesized and tested, since they should be less neurotoxic than any in current use and should reduce the incidence of neurological sequelae following carotid and spinal cord angiography.

(e) pH of the brain surface and the regulation of respiration. Acute pH changes at the cerebral cortex of the cat, after intravenous sodium bicarbonate or ammonium chloride, are related to changes in expiratory and blood pCO_2 rather than to changes in blood hydrogen ion, bicarbonate or ammonium concentrations. This is because the blood-brain barrier restricts exchange of polar solutes between blood and cerebrospinal fluid and because blood is a bicarbonate buffer system. An increase in cortical pH after NH_4Cl in spontaneously breathing animals is due to hyperpneic blowing off of CO_2 and not to entry of NH_3 into the cerebrospinal fluid, as suggested by the "Reaction Theory of Prespiratory Control," (Winterstein, 1956) and as found previously by Loeschcke & Sugioka (1969).

(f) Selective damage of ciliary epithelium of the eye by concentrated solutions, and breakdown of blood-aqueous barrier. We showed quite early that perfusion of hypertonic solutions into the internal carotid artery of monkeys led to breakdown of the blood-aqueous and blood-vitreous barriers to

protein tracers in the blood (Rapoport, S.I., D.S. Bachman and H.K. Thompson. Science, 176, 1243-1245, 1972). In order to determine the mechanism for these effects, Dr. T. Kuwabara, S. Okisaka and I studied the electron microscopy of the eye of the monkey in response to hypertonic perfusion, and related morphological changes there to changes in intraocular pressure. We found that hypertonic solutions damage the pigmented layer of the epithelium of the ciliary body, reduce the blood-aqueous barrier to proteins, and dramatically decrease intraocular pressure. Several months following hypertonic perfusion, the blood-aqueous barrier is restored and intraocular pressure has returned to its normal level.

Significance to Bio-medical Research and the Program of the Institute:

(a) The blood-brain barrier regulates exchange of materials between blood and brain and therefore controls brain metabolism and is important in brain pharmacology, since some drugs are excluded by it from entering the brain. Osmotic barrier opening is the first demonstration of a reversible opening that is based on morphology and simple physical-chemical laws applicable to cells in general. It will permit experimental modification of the blood-brain barrier for studying brain metabolism and perhaps for brain pharmacology. The theory of osmotic opening can be used to interpret regulatory functions of the barrier, and to understand how diseases associated with plasma hypertonicity can modify the barrier and produce clinical symptoms. These diseases include, for example, diabetic acidosis associated with coma.

(b) X-ray contrast media are injected into the carotid artery to visualize the cerebrovascular tree for clinical disorders of the brain. There is a significant morbidity and mortality associated with the technique, yet the factors affecting neurotoxicity are poorly understood. This work shows that (a) these agents can and probably do open the blood-brain barrier because of their high osmolality, and (b) that even if they do not open the barrier, they can enter the brain and affect neurones by virtue of their lipid solubility properties. This is the first time that neurotoxicity of X-ray contrast media is considered in terms of simple physical-chemical laws of osmosis and cell membrane permeability, and it would be hoped that less toxic contrast media (with lower osmolalities and lipid solubilities) will be synthesized in the future. On the basis of the relation between lipid solubility and neurotoxicity, we have been able to suggest a series of compounds which should be less neurotoxic than any in current use and should reduce the incidence of untoward effects in cerebral and spinal cord angiography.

(c) Regulation of respiration at the level of the central nervous system depends on the pH of cerebrospinal fluid and extracellular space of the brain. Previous work suggested that ammonia, produced by injection of ammonium chloride, also stimulated respiration centrally. This was the basis of the Reaction Theory of Respiration. We showed that this theory is partially incorrect and that the ammonium chloride effect on respiration is probably through changes in blood and brain CO_2.

(d) We have for the first time distinguished the role of the pigmented and nonpigmented epithelial layers of the ciliary body of the eye in regulating aqueous secretion. We have shown that the pigmented layer alone can control secretion and maintain normal intraocular pressure. The mechanism controlling pressure must be understood before successful therapy of glaucoma can be accomplished. Our findings that intraocular pressure can be reduced below normal over a period of months suggest that hypertonic infusion of the eye might be of eventual use in the treatment of acute glaucoma. It remains to demonstrate whether perfusion produces important irreversible changes in eye function.

Proposed Course of Project:

(a) I am writing a book entitled "The Blood-Brain Barrier in Physiology and Medicine," in which I extend my work and theory on osmotic opening of the blood-brain barrier, its importance in physiology and pharmacotherapy of the brain, and in which I go into detail on factors regulating drug entry into the brain.

(b) We shall continue our efforts to propose formulas of less toxic X-ray contrast agents for use in carotid angiography, and intend to begin work on trying to understand the mechanism of neurotoxicity at neuronal membranes.

(c) The effect of osmotic opening of the barrier on regulation of water and ionic contents of brain will be studied experimentally.

(d) We shall write up the work on the effect of hypertonic solutions on eye morphology and physiology.

Honors and Awards:

(1) Award for best paper presented at Eleventh Annual Meeting of American Society of Neuroradiology, April, 1973, Boston, Mass. To be published as award paper in American Journal

of Roentgenology and Radio-therapy, with title: "Dependence of Neurotoxicity of X-ray Contrast Media on Lipid Solubility."

(2) Invited Lecture: Harvard Medical School, Department of Pharmacology, October, 1973: "Reversible Osmotic Opening of the Blood-Brain Barrier."

(3) Invited Lecture: Cornell University Medical School, Department of Radiology, January, 1974: "Neurotoxicity of Radiographic Contrast Media."

(4) Invited Lecture: Pediatric Grand Rounds, Georgetown University Medical School, February, 1974: "The Blood-Brain Barrier and the Treatment of Meningitis in Children."

(5) Invited Lecture: Johns Hopkins University School of Medicine, Department of Physiology, March, 1974. "Reversible Osmotic Opening of the Blood-Brain Barrier and the Semipermeability Properties of the Barrier."

(6) Invited Participant: Alfred Benzon Symposium VIII, Copenhagen, Denmark, May 26-30, 1974: "The Working Brain. The Coupling of Function, Metabolism and Blood Flow in the Brain."

Publications:

Rapoport, S.I. and Thompson, H.K.: Osmotic opening of the blood-brain barrier in the monkey without associated neurological deficits. Science 180: 971, 1973.

Rapoport, S.I.: Evidence for reversible opening of the blood-brain barrier by osmotic shrinkage of the tight junctions. Relation to carotid arteriography. In Hilal, S.K. (Ed.): Small Vessel Angiography. Association of University Radiologists, April 23-26, 1972, Glen Cove, Long Island, New York. St. Louis, C.V. Mosby Company, 1973.

Brightman, M.W., Hori, M., Rapoport, S.I., Reese, T.S., and Westergaard, E.: Osmotic opening of tight junctions in cerebral endothelium. J. Comp. Neurol. 152: 317-326, 1973.

Rapoport, S.I. and Thompson, H.K.: Effect of intravenous NH_4Cl and $NaHCO_3$ on the pH of the brain surface, as related to respiration and the blood-brain barrier. Exp. Neurol. 42: 320-331, 1974.

Serial No. M-NP-63
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The role of cerebral motor cortex in control of movement

Previous Serial Number: Same

Principal Investigator: Edward V. Evarts

Other Investigator: Jun Tanji

Cooperating Units: None

Man Years:

Total:	4.87
Professional:	3.25
Other:	1.62

Project Description:

Objectives: This project is aimed at understanding how the inputs and outputs of the cerebral motor cortex function in control of movement and to discover what areas of the nervous system initiate motor cortex output, what areas control and regulate this output, and how these areas for initiation and control are interrelated.

Methods Employed: 1) Electrical Recording. Microelectrodes are used to record the activity of individual neurons during natural, voluntary movement in the monkey. This use of single unit analysis in relation to voluntary movement is analogous to the long-established use of single unit analysis in determining how the brain receives and elaborates its sensory input, but single unit analysis has only recently been carried out in animals trained to perform skilled movements during recording.

2) Behavioral Training. The current work on this project is concerned with the effects of behavioral set and expectancy on activity of neurons in sensorimotor cortex of the monkey. For this experiment, monkeys were trained to grasp a handle and maintain it in a certain position for 2 to 4 sec. After this 2-4 sec period, an "instruction" was given. This "instruction" told the monkey how he should move in response to a perturbation of the handle which would occur subsequently. The "instruction" was a red or green light which appeared after 2 to 4 sec of correct holding and 0.6 to 1.2 sec prior to the onset of a handle perturbation. The times varied unpredictably within these ranges. The red light informed the monkey that he should pull

toward himself when the perturbation occurred and the green light meant that he should push away when the perturbation occurred. There were two different directions of perturbation, one being a movement of the handle toward the monkey and the other a movement of the handle away from the monkey. A given instruction (red or green light) called for a movement synergistic with the tendon jerk and stretch reflex for one direction of perturbation and antagonistic to these reflexes for the other direction of perturbation. According to the hypothesis that modification of the stretch reflex by prior instruction involves a cortical loop, a change of stretch reflexes by prior instruction should be accompanied by a change in the short latency response of sensorimotor cortex neurons. When monkeys had learned to perform this task, recordings from individual sensorimotor cortex neurons were obtained during performance of the movement. Studies have been completed on more than 300 neurons in three monkeys.

Major Findings: In essence, three new observations have emerged from this experiment. First, it was found that short (20 msec) latency responses of motor cortex neurons can be gated on or off depending upon a prior instruction. The direction in which the gating takes place corresponds to the function of the motor cortex neuron in relation to the movement to be performed. Thus, if a particular motor cortex neuron is active in association with a pull movement, then a perturbation of the handle following the instruction to pull gives rise to a heightened reflex response in this neuron, and a perturbation of the handle following the instruction to push gives rise to a lessened reflex response in the neuron.

The second finding pertains to the tendon jerk. It was observed that this spinal cord reflex was modified by prior instruction: when the prior instruction called for activation of a particular muscle, the tendon jerk caused by stretch of this muscle was heightened, whereas when the prior instruction called for quiescence of a muscle, the tendon jerk to stretch of this muscle was suppressed.

The third observation was a modification of activity in motor cortex neurons by the prior instruction itself, such that by the time the perturbation occurred (0.6 to 1.2 sec after the instruction) the cerebral cortex was already in a different state depending on which of the two instructions had been given. It seems likely that the modification of motor cortex activity by instruction provides a pattern of descending impulses which underlies the presetting of spinal cord reflex mechanisms. The striking change of activity elicited by instruction was most marked in the precentral division of the sensorimotor cortex, but there were nevertheless some changes in postcentral cells.

Significance to Biomedical Research and the Program of the Institute: The results of this study show that a given peripheral input may generate quite different outputs from motor cortex depending on the movement called for by a prior instruction. Thus, modification of reflexes by prior instruction is associated with a change in motor cortex activity: "motor cortex reflexes"

as well as "stretch reflexes" can be gated by prior instruction. In addition to this observation on gating of motor cortex reflexes by prior instruction, two additional (and unexpected) findings emerged from this study. The first was that the tendon jerk, as well as the stretch reflex, is modified by prior instruction. A second unanticipated finding was the change of activity in motor cortex neurons in response to the instruction per se, with this response beginning at intervals of about 200 msec following the instruction. This change in motor cortex activity was not associated with changes in peripheral muscular activity. It seems clear that impulses descending to the spinal cord as a result of the modificiation of motor cortex activity set up 200 msec following the instruction might serve as a basis for the presetting of spinal cord reflexes. A number of the motor cortex neurons whose discharge changed in response to instruction were pyramidal tract neurons which might modify motoneuronal excitability directly or via the internuncial pool. It may also be conjectured that axons of some of these pyramidal tract neurons terminated on fusimotor neurons of the spinal cord and that these connections might represent a mechanism for changing the excitability of muscle spindles, thereby giving rise to the observed alternation of tendon jerks as a function of prior instruction.

The finding that nerve cells in sensorimotor cortex undergo specific changes as a function of central set and expectancy provides insight into the neuronal factors which may mediate selective attention, purposive behavior, and learning. The neuronal events which have been seen must involve changes in parts of the nervous system anatomically close to primary receptors, and this fact allows us to focus attention on a limited anatomical zone in seeking to discover where the changes may be taking place in association with modifications of behavior based on learning.

Proposed Course: The finding that the learned set of an animal can so profoundly modify patterns of activity in sensorimotor cortex leads us to try to localize the particular sites in the nervous system where these modifications may take place. Does modification of neuronal response to a peripheral stimulus take place as a result of changes in the thalamus or in the spinal cord? If we can find the site and then locate the particular neuronal processes which give rise to it, we will have made great advances in understanding the cerebral mechanisms underlying learning and selective attention.

Honors and Awards:

1. President-Elect, Society for Neuroscience

2. International Symposium, Centre National de la Recherche Scientifique, Marseille, September 7-9, 1973

3. Invited Lecturer, Biophysics Department, Leningrad State University, August 23, 1973

4. Participant, Neurosciences Work Session, Neurosciences Research Program, Boston, December 2-4, 1973

5. Invited Lecturer, USSR Academy of Sciences, Moscow, December 27-29, 1973

6. Sloan Lecturer, Department of Neurosciences, University of California, San Diego, April 25-26, 1974

7. Grass Traveling Lectureship, Society for Neuroscience, Seattle, Portland, Eugene, and Tucson, May 9-11, 1974

8. Invited Lecturer:

Center for Brain Research, University of Rochester, November 29, 1973
Department of Physiology, Virginia Commonwealth University, January 31, 1974
Department of Neurology, Cornell University, March 21, 1974
Department of Physiology and Pharmacology, Duke University, April 14-16, 1974
Department of Physiology, Downstate Medical Center, Brooklyn, April 30, 1974
School of Basic Health Sciences, State University of New York, Stony Brook, May 1, 1974
Department of Physiology, University of Rochester, May 2, 1974

Serves on:

1. Board of Co-editors, Experimental Brain Research

2. Advisory Board, International Journal of Neuroscience

3. Editorial Board, International Review of Neurobiology

4. International Research Review Panel, Fogarty International Center, NIH

5. W. T. Wakeman Award Panel, National Paraplegia Foundation

6. Council, Society for Neuroscience

7. Chairman, Task Force on Neurophysiology, American Physiological Society

Publications:

Evarts, E.V.: Pre- and postcentral neuronal discharge in relation to learned movement. In Frigyesi, T.L., Rinvik, E. and Yahr, M.D. (eds.): Corticothalamic Projections and Sensorimotor Activities. New York, N.Y., Raven Press, 1973, pp. 449-458

Evarts, E.V.: Brain mechanisms in movement. Sci. Amer. 229: 95-103, 1973

Evarts, E.V.: Sensorimotor cortex activity associated with movements triggered by visual as compared to somesthetic inputs. In Schmitt, F.O. and Worden, F.G. (eds.): The Neurosciences, Third Study Program. Cambridge, Mass., MIT Press, 1974, pp. 327-337.

Evarts, E.V. and Tanji, J. Gating of motor cortex reflexes by prior instruction. Brain Res. 71: 479-494, 1974.

Evarts, E.V.: Precentral and postcentral cortical activity in association with visually triggered movement. J. Neurophysiol. 37: 373-381, 1974.

Serial No. M-NP-65
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Functional role of sub-cortical inputs to the motor cortex in the control of movement

Previous Serial Number: Same

Principal Investigators: Peter L. Strick

Other Investigators: Mahlon R. DeLong

Cooperating Units: None

Man Years:

Total:	2.98
Professional:	1.75
Other:	1.23

Project Description:

Objectives: This study is directed towards learning how sub-cortical nuclei function in the control of movement and posture. In particular, the types of movement they control and the sorts of information they provide the motor cortex through cortical afferent pathways orginating in the ventrolateral nucleus of the thalamus (VL) are being (has been) investigated.

Methods Employed: Micro-electrodes are used to record the activity of individual neurons during trained voluntary movement in monkeys, a technique developed in this laboratory over the past years. Monkeys are trained to perform skilled movements and cell activity is correlated with various aspects of the movements.

Major Findings: In monkeys trained to execute both slow and rapid arm movements, it was found (DeLong, Science 179: 1240-1242, 1973) that more than half of the movement related units recorded in the putamen discharged preferentially in relation to slow movements while only a few discharged preferentially during rapid movements. These findings were interpreted as lending experimental support to the view of Kornhuber (Kybernetic 8: 157, 1971) that the basal ganglia function primarily in the generation of slow movements. In the present

study unit activity was recorded from the globus pallidus as well as the putamen, in order to determine whether the preferential relationship of units for slow movements is also found in this nucleus, which gives rise to the major output from the basal ganglia. Confirming the earlier observation, the majority of movement-related units in putamen discharged preferentially during slow movements. In the globus pallidus also, 17% of all movement-related units discharged preferentially during slow movements, and only a small number preferentially during rapid movements. In contrast to units in the basal ganglia nearly all of the movement related units in the motor cortex and cerebellum discharged in relation to both slow and rapid limb movements. Taken together, these findings lend further support to the hypothesis of a preferential role of the basal ganglia in the control of slow movements.

In another set of experiments the activity of VL neurons was recorded in awake monkeys trained to maintain the handle of a manipulandum within a small zone, despite perturbations to handle, and to perform slow and rapid arm movements triggered by a visual stimulus.

VL units related to arm movement were located within a restricted medio-lateral zone centered at approximately the middle of the nucleus; and neurons in this zone discharged in relation to both slow and rapid arm movements. Thus VL neurons are unlike one source of its afferents, the globus pallidus, which contains neurons that are preferentially related to slow movements.

Units related to jaw and tongue movements associated with the juice reward were located medial to arm related units. Concurrent anatomical studies utilizing retrograde transport of horseradish peroxidase indicate that the VL region in which units related to arm movement were recorded projects to the "arm" area of the motor cortex. In addition microstimulation (< 25 μA) in the arm movement related region of VL evoked contractions of shoulder and forelimb musculature.

Unlike hand area motor cortex neurons, most VL neurons showed little or no change in activity in relation to passive manipulation of arm or hand. Some VL neurons, however, did show short latency (< 50 msec.) changes in activity following small perturbations of the manipulandum handle held by the monkey. Whether these short latency VL responses reflect sensory driving during task performance or short latency motor responses related to the corrective movements which followed the handle perturbation remains to be determined.

Significance to Bio-medical Research and the Program of the Institute: Experimental and clinico-pathologic studies in both animals and man have long indicated that the basal ganglia and cerebellum play an important role in the control of movement and posture. Abnormalities in movement, posture and involuntary movements frequently result from damage to these structures produced either experimentally or through disease.

The outputs of the basal ganglia and the cerebellum converge on the ventrolateral nucleus of the thalamus (VL) which is the major source of subcortical input to the motor cortex. It is the combination of thalamic and cortical inputs which determines the output of the motor cortex. Thus the thalamus plays an important role in the initiation and control of movement by processing and relaying the outputs of the major subcortical motor nuclei to the motor cortex. Analysis of the function of the basal ganglia, cerebellum and thalamus in movement control have previously relied largely on the techniques of stimulation and ablation, or cell recording in anesthetized animals. This project applies the techniques for single cell recording in awake-trained monkeys in order to learn more about how these subcortical structures normally function during movement.

It has been suggested that in a number of movement disorders the thalamus plays a prominent role in propagating the abnormal signals of diseased subcortical structures to the motor cortex. For this and other reasons thalamic lesions have been made in human patients for a number of years to reduce the tremor and rigidity accompanying Parkinson disease. The discovery and use of L-Dopa drug therapy has reduced the use of thalamic-lesion treatment; however, many neurologists maintain that the best results are obtained with the combination of both drug therapy and thalamic lesions. The present project should provide a firmer basis for the treatment of brain disorders causing abnormal movements by furthering our understanding of what role the thalamus plays in the control of normal movement.

Proposed Course of Project: Experiments now in progress are concerned with further defining the type of information the thalamus provides the motor cortex. The questions which will receive the most attention will be: 1) Do thalamic neurons provide the motor cortex with information on what events have transpired in the periphery (sensory role)? or 2) Do thalamic neurons provide the cortex with motor commands generated in subcortical structures (motor role)?

In addition, experiments will test the contribution of the thalamus to different types of movement by lesioning it and recording the changes in the animal's ability to perform motor

tasks. Finally single units may be recorded in the motor cortex following thalamic lesions to determine what information which normally appears in the motor cortex is then absent.

Honors and Awards: None

Publications:

DeLong, M.R. and Strick, P.L.: Relation of basal ganglia, cerebellum, and motor cortex units to ramp and ballistic limb movements. Brain Res. 71, 1974.

Serial No. M-NP-66
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: The role of the vestibular nuclei in the generation of head and eye movements

Previous Serial Number: Same

Principal Investigator: F. A. Miles

Other Investigators: James Fuller

Cooperating Units: None

Man Years:

Total:	4.00
Professional:	2.75
Other:	1.25

Project Description:

Objectives: The vestibular nuclei receive sensory information from the labyrinth which accurately describes both the orientation of the head in space and also any rotary motion which it undergoes; this information is needed for the maintenance of body posture and is vitally important in the stabilization of the head and eye. Experiments were designed to establish whether the vestibular nuclei are solely sensory relay areas or whether they also participate directly in the generation of the motor programmes which ultimately guide the eyes, head and trunk.

Methods Employed: The approach used here involved microelectrode recordings in the vestibular nuclei in conscious monkeys, and initially concentrated on individual units whose firing patterns correlated with the animal's saccadic eye movements. Each animal was trained to press a bar which switched on a small spot of light on a screen facing him and to release the bar only after the light dimmed. If the animal succeeded in releasing the bar within 1/2 sec. of this dimming, then he was rewarded with a drop of water; all of the animal's fluid intake was earned by working at this task. Using this approach, monkeys were trained to fixate a small spot of light and maintain

this fixation even if the position of the spot was changed. Thus, it was possible to induce the animal to generate saccadic eye movements of known magnitude and direction merely by changing the position of the fixation target. This behavioural task requires immense vigilance from the animal and cannot be performed with peripheral vision, hence successful performance could only be achieved by the animal if he made the required eye movements. Oculograms recorded from implanted electrodes confirmed the nature of the animal's eye movements whilst he was performing the task and were used as a response trigger for data display: correlations between eye movements and individual neurone firing patterns were made on-line by using a raster display in which each action potential was represented as a spot on the screen of a storage oscilloscope; the oculogram signals generated by the eye movements were used to initiate sweeps of the oscilloscope and the pulses representing action potentials were displayed after passing through a delay line. In this way, unit firing patterns during successive saccade trials were displayed in rows on the storage oscilloscope; the delay facility allowed one to observe the unit firing before, as well as during and after, the eye movements. A major advantage of this form of display is that it readily reveals not only the nature of the correlation between eye position and unit firing but also its timing and consistency.

Upon isolating an eye-movement correlated unit, the animal was induced to make a variety of eye movements with various amplitudes, directions, and start position. Previous studies by the present investigator had shown that the activity of a large proportion of the cells in the medial and superior vestibular nuclei is very closely correlated with eye movements. Some neurones fire in relation to the position of the eyes in the orbits, others only in relation to the movement of the eyes, and yet others show both relationships; occasional units show maintained firing which is suppressed during all eye movements. Thus, each saccade is accompanied by a step, pulse, or pulse-step change in the firing of individual neurones. The precision of this coupling between single neurone behaviour and eye movements is especially impressive and the vestibular nuclei are clearly in possession of very accurate information about the oculomotor programmes. In some units the earliest changes in firing precede the eye movement by 10-20 msec. whilst in others they occur during the movement. Many neurons are strongly directional, firing only in relation to movements of the eye to one or other side of the orbits, whilst others--especially the pulse-type units--often fire in relation to all eye movements.

The present experiments were designed to examine the behaviour

of these eye-movement correlated neurones in a variety of behavioural paradigms to see if there were any circumstances under which their activity could be dissociated from eye movements. To this end, monkeys were induced to generate various patterns of eye movements whilst being oscillated to and fro about a vertical axis in a special servo-driven chair. These rotations are sensed by the animal's vestibular system which generates compensatory eye movements and hence serves to maintain a reasonably stationary retinal image.

During the course of these experiments, it became clear that some "burst-tonic" neurones did not always fire solely in relation to movements of the eyes in the orbits. Three experimental paradigms were employed in the analysis of these neurones, and it is important to note that the animal's head was secured to the special primate chair throughout: (1) The chair was stationary, and the animal endeavoured to track a target which oscillated back and forth; this was an attempt to obtain a sinusoidal pattern of horizontal eye movements. (2) The chair was oscillated about a vertical axis while the animal fixated a distant stationary target; this was an attempt to obtain a pattern of eye movements similar to those produced in the previous situation, but now by moving the animal instead of the target. (3) The chair was oscillated exactly as in (2), but the monkey's fixation target was mounted on a bracket extending from the chair and hence moved with the animal; here, the monkey had to try to suppress the compensatory eye movements which normally accompany such head rotations.

Major Findings: Most of the eye-movement correlated neurons fired purely in relation to the movements of the eyes in the orbits no matter what experimental conditions prevailed. However, the activity of some of the "burst-tonic" neurons could be dissociated from eye-movements. This was seen most dramatically when the fixation target was oscillating with the chair so that the monkey's eyes were reasonably stationary in the orbits. Under such conditions these neurons continued to modulate their activity albeit now in association with the chair oscillations. It was felt that the apparent eye-movement relationships seen in such units when the animal's head was fixed in position may in fact have been associated with attempted head movements.

The possibility that the animal was indeed trying to move its head even though its skull had always been securely bolted to the chair throughout all recording sessions was examined by recording the monkey's head torque through strain gauges applied to the head mount. It was clear that eye movements were often accompanied by attempted head movements and the activity of some neurons was closely correlated with and preceded these attempted

head movements. Perhaps most interesting of all was the finding that many of the neurons which had previously been regarded as "purely vestibular" because they modulated nicely in association with chair velocity, were observed to fire in association with the attempted head movements when the chair was stationary; such neurons would accelerate their firing as the chair moved towards one or other side and, without exception, also increased their firing whenever the monkey attempted those head movements which would have carried the head in the opposite direction.

Significance to Bio-medical Research and the Program of the Institute: It is now clear that the activity of many neurons in the vestibular nuclei closely reflects various elements of the oculomotor programme under a wide variety of behavioural conditions. This finding strengthens the idea that all eye-movement programmes are "compiled" in the vestibular nuclei. In addition, it is now evident that even animals whose heads have been habitually immobilized in the experimental situation by bolting still attempt some head movements. Such gestures are often tightly coupled to eye movements and the neural events in the vestibular nuclei often reflect various aspects of such centrally programmed head movements. The present study also showed that many of the neurons which have classically been called "vestibular" because they seemed to be driven purely by head rotation in a particular direction, also fire when the animal actively attempts to turn its head in the converse direction; this strongly suggests that given neurons in the vestibular nuclei mediate both vestibularly induced and centrally programmed contractions of the neck muscles.

Proposed Course of Project: The next part of the chronic single unit recording project will seek to characterize further the behavior of those neurons in the vestibular nuclei which seem to relate to both the labyrinthine input and the centrally generated head movement commands. This will necessitate the development of further behavioural paradigms to fractionate these two factors. In addition preliminary recordings will be made in some areas known to provide major inputs to the vestibular nuclei (e.g. Interstitial Nucleus of Cajal and the Flocculus) and others known to receive a projection from these nuclei (e.g. Vestibular cortex and cerebellum) which have been implicated in head and/or eye movement programming by other workers.

Preliminary experiments by the present investigators have shown that when monkeys wear telescopic spectacles for a few days, the gain of their vestibulo-ocular reflex can be increased by at least 50%. This represents a very clear-cut example of adaptive plasticity and it is proposed to follow up these initial findings

with the hope of characterizing the underlying mechanisms.

Honors and Awards:

1. Invited to give a paper at an International Symposium on "Comportement moteur et activites nerveuses programmees", in Aix-en-Provence, France - Sept. '73.

2. Co-chairman of a Neurosciences Research Program Workshop on "Current problems in behavioural neurophysiology" in Boston, USA - Dec. '73.

3. Invited to give two seminars to the Northwestern Chapter of the Society for Neuroscience, in Seattle and Portland, USA - March '74.

Publications:

Miles, F.A.: Single unit firing patterns in the vestibular nuclei related to voluntary eye movements and passive body rotation in conscious monkeys. Brain Res. 71: 215-224, 1974.

Serial No. M-NP-68
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the neuronal activity of the prefrontal cortex

Previous Serial Number: Same

Principal Investigator: Hiroaki Niki

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.25
Professional:	0.25
Other:	

Project Description:

Objectives: An improved understanding of the prefrontal cortex has long been a major research goal within a number of different subdisciplines within the field of neuroscience. In particular, research workers in the clinical disciplines of neurology and psychiatry and the experimental disciplines of neurophysiology and psychology have sought to advance knowledge of the function of the prefrontal cerebral cortex in man and subhuman primates. The goal of the present project has been to achieve a more detailed understanding of the organization of the neuronal activity occurring in association with behavioral tasks in which prefrontal cortex is critically involved.

Methods Employed: In the present studies using subhuman primates, our point of departure comes from the fact that lesions of the prefrontal cortex give rise to marked disturbances in spatial memory. These defects in spatial memory following prefrontal cortex lesions in subhuman primates have been assessed by performance on tasks referred to as "delayed alternation" or "delayed response." In these tasks the monkey subject is required to remember which of two spatially separate locations he should select. For the present study, delayed alternation was used. For such a task the animal is required to depress a central key for a period of several seconds, following which two laterally placed keys become illuminated. The monkey must then select the key he did not select on the previous trial. On successive trials he must select these two laterally placed keys in alternation. Thus, he must first depress the central key and when the laterally placed keys become illuminated he must press the one to the right. He then returns to the central hold key,

but the next time the laterally placed keys become illuminated he must press the left key. This task continues for many trials. In association with performance of this task the present project involved recording the activity of single nerve cells in prefrontal cortex by means of microelectrodes.

Major Findings: The major findings from this project may be divided into two sections.

1. Prefrontal unit activity during delayed alternation in the monkey. I. Relation to direction of response. Single unit activity was recorded from the area midprincipalis of the dorsolateral prefrontal cortex during delayed alternation (DA) in one monkey. Out of 502 units studied in both hemispheres, 198 showed a relation to aspects of the DA task. Half of these related units showed a differential firing pattern depending on the trial (left or right). In 26 units the discharge frequency during delay was different between right and left trials. In no case, however, was such selectivity during delay found in a cue-guided control task.

The finding that a significant proportion of neurons in the prefrontal cortex is differentially active during the delay period depending upon whether the selection to follow will be to the right or to the left demonstrates that activity of prefrontal cortex cells can code the spatial memory which is necessary to guide the monkey's impending motor response. This is the first demonstration of this fact.

2. Prefrontal unit activity during delayed alternation in the monkey. II. Relation to absolute versus relative direction of response. Single unit activity was recorded from the area principalis of the dorsolateral prefrontal cortex during DA in three monkeys in order to analyze further the nature of observed differential changes in relation to the different positions of the choice keys (directional selectivity). Out of 354 related units obtained from 170 penetrations, 182 showed directional selectivity. A total of 111 directionally selective units were investigated in sufficient detail to determine whether the observed directional selectivity was related to the absolute or to the relative position of the choice keys.

Twenty of these 111 units showed directional selectivity during the delay period and all of these units showed clear dependence on the relations between choice keys. For the remaining units, whose directional selectivity became apparent only after the choice keys were illuminated, dependence on relative factors was less marked. All units showed some dependence on the location of the choice keys, independent of the relations between choice keys.

This finding on the significance of the relative position of the choice key indicates that an important dimension of coding of spatial memory in prefrontal cortex is the "rightness" or "leftness" of the choice key. This indicates that spatial memory is coded at a rather high level of abstraction in the prefrontal cortex of the primate brain.

Significance to Biomedical Research and the Program of the Institute: The prefrontal cortex is a region of the brain which is a borderland between neurology, psychiatry, neurophysiology, and neuropsychology. The present project has succeeded in revealing certain aspects of the way in which spatial memory is coded in this part of the brain. Much remains to be done in connection with this type of work, but the present work has made a beginning with its discovery that nerve cells in the prefrontal cortex show directional selectivity during a delay period and thus that they may participate in a central mnemonic code for correct spatial choice.

Proposed Course: The principal investigator, Dir. Hiroaki Niki, has left the United States and is currently working at the Tokyo Metropolitan Institute for Neurosciences. He is continuing work on the prefrontal cortex of the primate at his laboratory in Japan and will pursue a number of questions which need to be answered in order that we have a more thorough understanding of the function of prefrontal cortex.

Honors and Awards: Upon returning to Japan Dr. Niki became the chief psychologist in the new Brain Research Institute in Tokyo

Publications:

Niki, H.: Prefrontal unit activity during delayed alternation in the monkey. I. Relation to direction of response. Brain Res. 68: 185-196, 1974.

Niki, H.: Prefrontal unit activity during delayed alternation in the monkey. II. Relation to absolute versus relative direction of response. Brain Res. 68: 197-204, 1974.

Niki, H.: Differential activity of prefrontal units during right and left delayed response trials. Brain Res. (in press).

Serial No. M-NP-69
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Influence of centrally-acting drugs on motor cortex activity during attentive behavior

Previous Serial Number: Same

Principal Investigators: Jesus B. Otero and Allan F. Mirsky

Other Investigators: None

Cooperating Units: Division of Psychiatry
Boston University
School of Medicine
Boston, Massachusetts

Man Years:

Total:	3.07
Professional:	2.00
Other:	1.07

Project Description:

Objectives: The overall goal of this research is to understand the central nervous system alterations occurring during impaired attentive behavior. Previous work has investigated behavioral and EEG aspects of impaired attention produced in trained monkey subjects by the administration of secobarbital and chlorpromazine (cf Bakey Pragay, E. and Mirsky, A. F.: The nature of performance deficit under secobarbital and chlorpromazine in the monkey. Psychopharmacologia (Berl.) 28, 73-85, 1973). This work suggested that the mechanism of impairment produced by these two drugs differed; chlorpromazine seemed to produce primarily a phasic blocking of input process subserving arousal. Secobarbital, on the other hand, seems to suppress the functional level not only of arousal processes but of motor effector mechanisms as well. The present study was designed to focus on these effector mechanisms as they come into play during the performance of a sustained discriminated attention task. The question being investigated is whether and how neuronal activity is altered by centrally-acting drugs during attentive behavior. The methods used permit dissection of two components of the motor behavior: time to initiate the response after the "go" signal has appeared (Initiation Time) and time to execute the response (Movement Time).

Methods Employed: Monkey subjects are trained to depress a "hold" key for a period of 2 seconds, following which either a red or a green cue lamp came on. The red lamp requires the hand to be moved from the hold key to a target button within 1 second of the cue presentation, whereas the green lamp requires continued depression of the hold key for 1 second following presentation of the green cue. Correct performance is reinforced with fruit juice. Recordings of extracellular motor neuron activity and both EEG and EMG are obtained during the performance of the task, and selected doses of centrally-acting drugs are administered. In addition, visual evoked potentials are recorded during interperformance periods. The intent is to monitor the activity of task-related neurons both prior to, and after drug administration. Ideally, single neurons will be followed during a complete cycle of drug action until behavioral recovery (i.e., pre-drug performance levels) is observed.

Major Findings: Data collection for the first stage of the experimental project has been completed. Analysis of chlorpromazine effects is not yet complete, but the following observations from neurons monitored during secobarbital administration can be noted:

1. As in the case of prior studies, significant increments in Initiation Time (IT) are observed with a parallel occurrence of errors of omission, at doses between 5 and 17.5 mg./Kg.

2. Changes in Movement Time (MT) depend on the dose: Low doses (5 to 10 mg./Kg.) produce decrease, and higher doses (10 to 17.5 mg./Kg.) produce increase of MT.

3. The occurrence of commission errors is not significant either at low doses or in most experiments in which higher doses were administrated.

4. No modulation of unit firing has been found as a consequence of "no-go" green stimulation.

5. In analysis of the responses to the "go" red stimulus it was possible to fractionate IT into two components: SF, the interval from the "go" signal (S) to onset of neuron firing (F), and FR, the interval from firing (F) to the first motor response (R). In this fractionation IT = SF + FR. Increases in IT were found to be a function of an increase in SF, rather than in FR, since this last interval was increased, in more than a relative 25%, only in 10% of the studied neurons.

6. Onset of firing of units for which FR interval increases under drug, showed shorter latency after stimulus presentation than units which FR interval increases under drug effect.

7. Neurons with shorter latency firing appear to be located in a more medial position into precentral gyrus than neurons with longer latency firing.

8. Recording of visual evoked potentials showed an increase in first components latency as well as an increase in amplitude.

Significance to Bio-medical Research and Program of the Institute: The information obtained from this project is relevant to two questions of interest to the Institute: (1) the symptom of attention impairment is prominent in several diseases of largely unknown pathophysiology, i.e., schizophrenia and petit mal epilepsy. Further, it is a commonly observed characteristic of hyperkinetic children. Any information concerning alterations in central nervous system activity during impaired attention should aid in the understanding of the possible brain pathology which is associated with these clinical problems. (2) Although heroin abuse receives more attention, barbiturate abuse is probably more widespread and more dangerous from a physiological point of view. Information concerning the neural effects of barbiturates and related compounds is needed to aid in the comprehension of this problem.

Proposed Course of Project: Additional animals will be prepared for study of barbiturate and chlorpromazine effects on neuronal activity. Results reported here as well as findings from different authors (Bucher and Burgui, 1952; Kuypers, 1966; Myers, 1964; Delgado, 1965; Kadobayashi, 1973; Barkay and Mirsky, unpublished data), strongly suggest that the present work should be extended to other regions of the Central Nervous System, particularly to the Red Nucleus, which has never previously been studied in behaving animals.

Honors and Awards: None

Publications: None

Serial No. M-NP-70
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Neurochemical and neuroanatomical correlates of behavior

Previous Serial Number: Same

Principal Investigator: Frank R. Sharp

Other Investigators: Rufus Gunn

Cooperating Units: None

Man Years:

Total:	2.00
Professional:	2.00
Other:	

Project Description:

Objectives: This project is aimed at obtaining neurochemical and neuroanatomical correlates of central nervous system activity occurring in association with behavior. Such information could provide valuable correlates for the microelectrode techniques in use in the laboratory. Microelectrode techniques are limited because they selectively record activity from a small set of large cells. Ideally, chemical changes of smaller neurons, neuronal processes, and glia should eventually be related to a behavioral state.

Methods Employed: To implement the above objective several techniques will be used. The first has been developed by Louis Sokoloff and his associates. This is an autoradiographic technique in which radioactive deoxyglucose is used as an indicator of glucose uptake of localized cerebral regions. Behavioral changes will be correlated to regional changes in glucose uptake. This may show anatomical localization of metabolically active areas and cells, interrelationships between active and inactive areas, and provide basic biochemical data on cerebral metabolism. Future projects would include: determining routes of entry, major determinants of uptake, drug effects, relations to disease, and subcellular localization of deoxyglucose. The second method has not been developed but appears promising.

This involves determining the amount of protein and RNA formed in a neuron. If the total RNA and protein content of the cell is related to behavior then this technique could provide an indicator of "active" neuronal cell bodies.

Major Findings: The findings over the last year have been mainly technical in nature. However, several preliminary experiments do suggest the techniques will serve as correlates of behavior. Stimulation of the sciatic nerve in an anesthetized rat increased the glucose uptake in one dorsal horn of the spinal cord several times the control side. In these experiments it was seen that most, if not all, elements of the central nervous system take up glucose but the exact proportions to assign to each await improvement in the technique. In addition, it has been found that the metabolic rate of the central nervous system of the swimming rat is significantly increased over the resting control rat.

Significance to Bio-Medical Research and the Program of the Institute: The project, if successful, would provide a different approach to investigating the central nervous system, one which would open new vistas into brain function and possibly malfunction. The techniques are to be designed to give an index of activity and be correlated to behavioral, pathologic and pharmacologic states of the central nervous system. The project would provide an eventual link between the Laboratories of Cerebral Metabolism, Neurophysiology, and Neuropharmacology.

Proposed Course of Project: The deoxyglucose technique is to be further developed to improve localization. A continuing attempt will be made to develop a cell body indicator of behavioral activity. With these techniques the metabolism of the central nervous system will be investigated in resting rats compared to running rats, young and old sleeping rats, and rats in many other physiological states. In addition, trained monkeys will be utilized to correlate the metabolism of different cerebral regions to a behavioral task.

Honors and Awards: None

Publications: None

Serial No. M-NP-71
1. Laboratory of Neurophysiology
2.
3. Bethesda, Maryland

PHS-ADAMHA-NIMH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Anatomical analysis of thalamic input to the motor cortex

Previous Serial Number: None

Principal Investigator: Peter L. Strick

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	

Project Description:

Objectives: This project is aimed at anatomically determining which regions of the thalamus influence the motor cortex and how the connections between thalamus and cortex are functionally organized.

Methods Employed: This study utilizes the phenomenon of "axon flow" to label pathways going into or out of the motor cortex. Radioactive labelled amino acids injected into the motor cortex are taken up and incorporated into proteins at the cell bodies of motor cortex neurons. These proteins are then transported from the cell body, down the axon, to their synaptic terminals (orthograde axonal flow). When the tissue is processed by autoradiographic methods, labelled proteins appearing in axons and synaptic terminals of motor cortex output pathways are visualized.

Low molecular weight proteins injected into the motor cortex are taken up by synaptic terminals in the motor cortex. These proteins are then transported from the terminals, up the axon, back to the cell body (retrograde axonal flow). When the tissue is processed by the appropriate histochemical methods, the protein appearing in cells which send their axons to the motor cortex is visualized.

Major Findings: Previous anatomical investigations concerning the thalamic projection to the motor cortex have presented conflicting results. Some studies indicate that a wide region of the thalamus projects to the motor cortex and that the projection is diffusely organized. Other studies suggest a well-ordered input, largely from the ventrolateral nucleus of the thalamus (VL). The aim of the present study has been to investigate the anatomical organization of thalamic input to the motor cortex in some detail.

Injections of horseradish peroxidase into the arm area of the motor cortex and its subsequent retrograde transport to the thalamus have successfully labelled the "arm area" of the thalamus, or more correctly the region of the thalamus which sends fibers to the arm area of the motor cortex.

Neurons which send fibers to the arm area of the motor cortex occurred within a restricted region of VL. Thalamocortical neurons were most concentrated at anterior 8.5, where they formed a "slab" of neurons, 6 mm long in the dorso-ventral direction, and 1 1/2 mm wide. The thalamic region which projects to the motor cortex did not also project to the sensory cortex, since no labelled neurons were found in VL following injections of protein into the sensory cortex of another set of animals.

These anatomical findings agree with physiological studies which are being carried out concurrently. Neurons which are well-related to arm movement are recorded in the VL region which projects to the motor cortex arm area, and electrical stimulation through the recording electrodes (< 25 μA) evokes contractions of arm musculature in this same region.

It is most interesting that in the VL region where the densest input to the motor cortex originates, most of the VL neurons are labelled. Thus there appear to be few interneurons in VL. Furthermore the labelled VL neurons vary greatly in size. If cell size in VL is correlated with axon size, as it is in other brain regions, then there should be a wide range of conduction velocities of thalamocortical fibers to the motor cortex.

Significance to Bio-medical Research and the Program of the Institute: The ventrolateral nucleus of the thalamus VL is the primary source of thalamic fibers to the motor cortex. Physiological studies indicate that the type of information the motor cortex receives is determined to a great extent, by the input from VL and thus the connection between VL and the motor cortex is a very important one. There is little information, however, about the anatomical organization of VL fibers through which

the VL influence on the cortex is exerted. The present findings are important because they outline a discrete anatomical system which plays an important role in controlling the information presented to the motor cortex and thus movement in general. The thalamus has been and will continue to be studied intensively with physiological methods. In trying to understand movement programming in the motor cortex and in the "motor" thalamus it will be most important to compare regions of both structures which are interconnected. The present studies indicate which are the relevant thalamic regions and provide a miniature road map for their localization.

Proposed Course of Project: Experiments are now in progress to determine: 1) Is the connection between the motor cortex and thalamus reciprocally organized? This will be determined by utilizing retrograde and orthograde axonal flow from the same site. The arm area of the motor cortex will be injected with both a protein (to determine the input to the region) and a labelled amino acid (to determine the output of the region). 2) Do separate regions of VL influence proximal vs distal musculature? 3) What is the axonal branching pattern of VL neurons? There is some physiological evidence that a special class of branching neurons exist in VL. These neurons have axons which are said to project to widespread regions of the motor cortex and thus might play a role in coordinating body movements.

New techniques are being developed to determine whether a single neuron projects to two different cortical regions and assess this possibility.

Honors and Awards: None

Publications:

Strick, P.L.: Light microscopic analysis of the cortical projection of the thalamic ventrolateral nucleus in the cat. Brain Res. 55: 1-25, 1973.

Strick, P.L., Hand, P.J., and Morrison, A.R.: Organization of thalamic projections to the motor cortex of the cat. In Frigyesi, T., Rinvik, E., and Yahr, M.D. (Eds.): Cortico-thalamic Projections and Sensorimotor Activities. New York, Raven Press, 1972, pp. 115-124.

Strick, P.L.: Synaptic termination of afferents from the ventrolateral nucleus of the thalamus in the cat motor cortex. A light and electron microscope study. J. Comp. Neurol. 153: 77-105, 1974.

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